The future of Chromatography materials and Preparative Chromatography

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Phenomenex

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Patent Pending

High Speed, High Throughput Preparative Chromatography

- Typical chromatographic conditions
  - -Low aspect ratio columns
  - -High flow rates — 20, 40 or 60mL/min
  - -High viscosity samples — 20-50 mg in 0.5-1mL
- Significantly increased stress on column packing
  - -Rapid column performance decay
  - -Column-to-column lifetime variation

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Limitations of Conventional Slurry Packing

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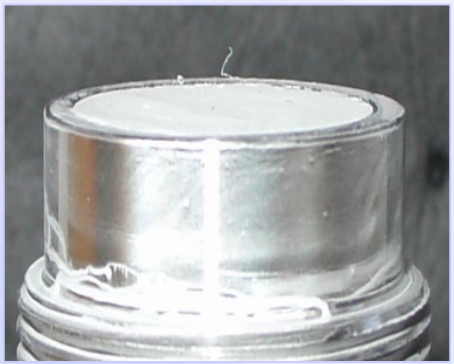
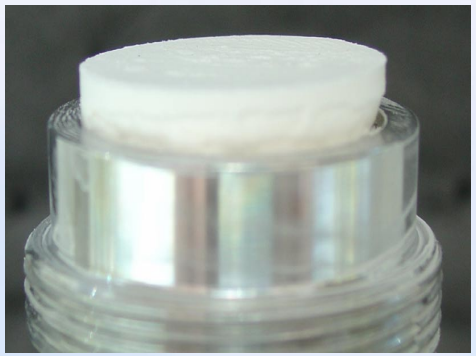
High pressure solvent forces sedimentation of the slurry

Disassembly of slurry chamber



Bed "relaxes" and extrudes



Lower density at the "uncapped" end

Non-uniform density

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Axia™ Packing Process

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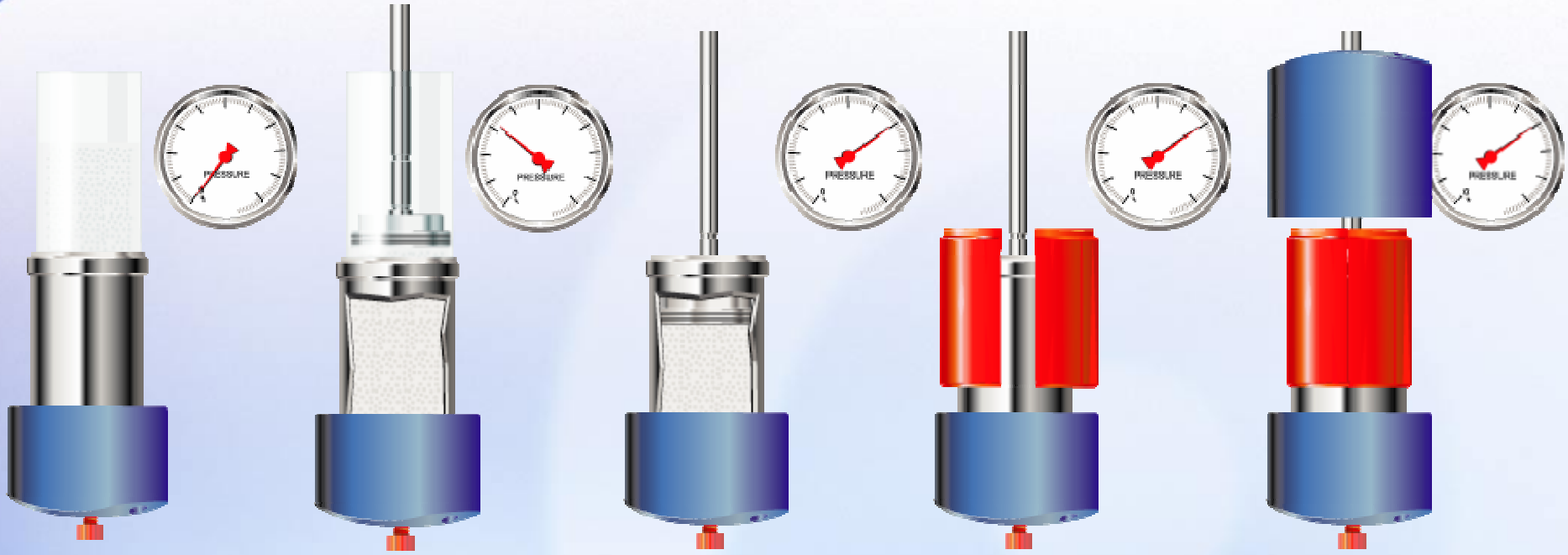

Media Added

Hydraulic Piston Compresses Bed

Column Bed Formed

End Fitting Attached

Retainer Sleeve Attached

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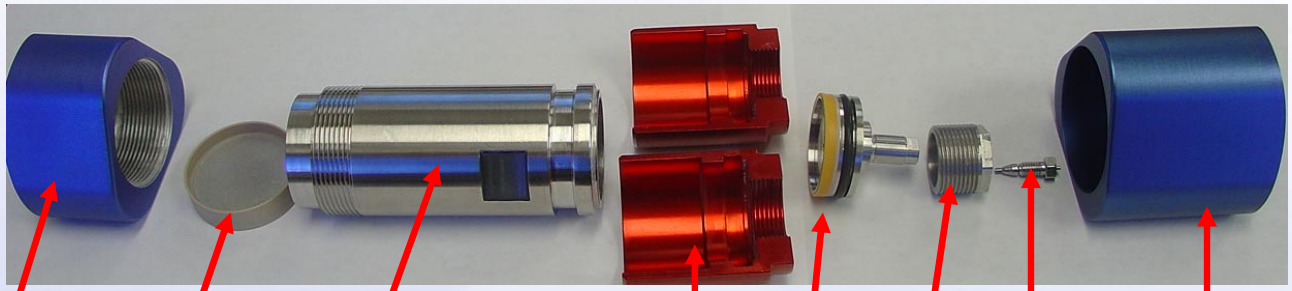
HPC™ Column Hardware – Retainer Sleeve Attached

"Never opened,

Never released"

Anatomy of the HPC™ Column Hardware



Retainer sleeve

Stainless steel plug

Bed compression screw

Piston sub assembly

Column tube

Cap frit

Piston retainers

End cap

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Vastly improved process control

- Micro-processor controlled media compression system
- Packing method completely automated
- Multiple sensors and linear encoders allow measurement and recording of all process parameters for every column

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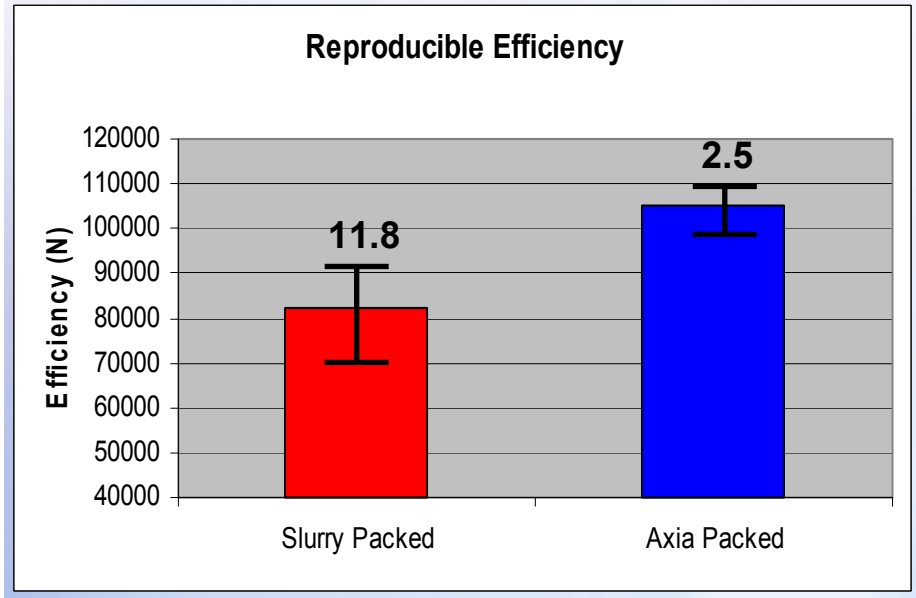
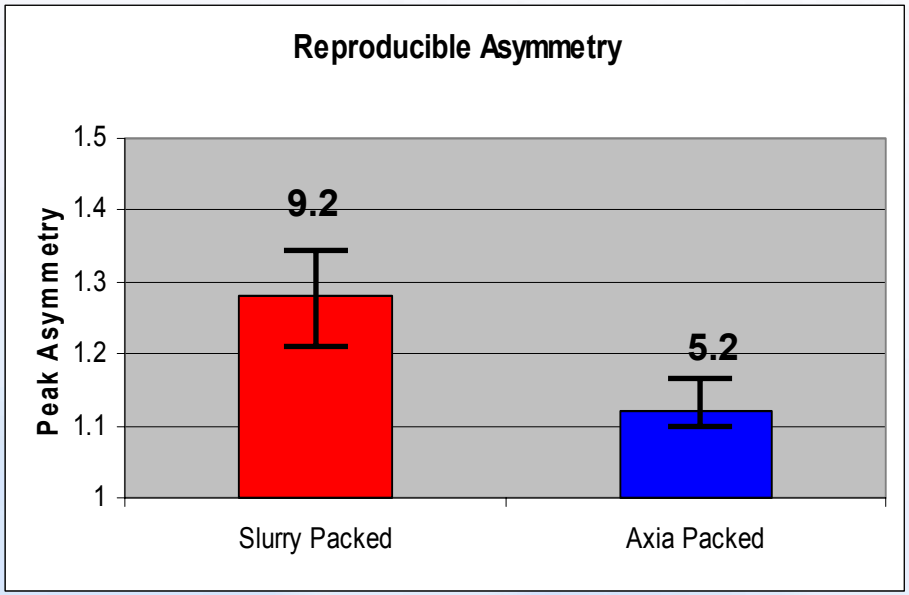
Axia™ Packed Columns: Improved Performance and Reproducibility

Summary of Eight Different Media





- Average efficiency increased by 27%
- Efficiency %RSD improved 4x
- Peak asymmetry improved by 13%
- Asymmetry %RSD decreased 2x

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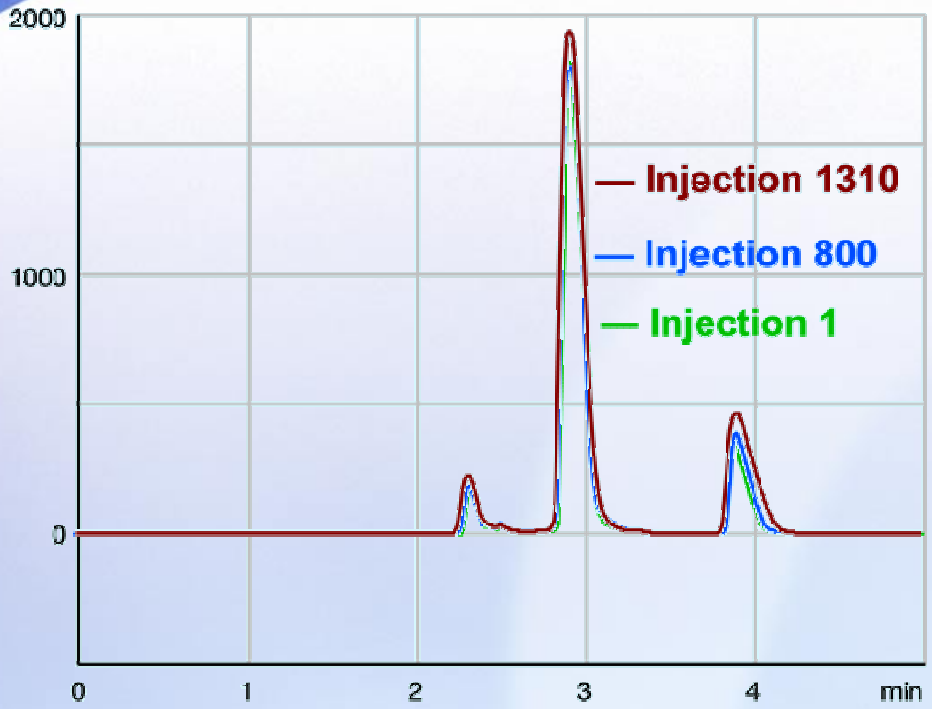
Axia™ Luna® C18(2) 5µm Gradient Lifetime Study



- Same preparative separation after 1, 800, and >1300 cycles
  - Less than 1.5% change in performance

Column: Luna® C18(2) 5µm 50 x 21.2mm
Flow Rate: 30mL/min
Gradient: 5 min 95:5 to 5:95 H_2O/MeCN with 0.5% TFA
Detection: UV @ 254nm
Injection volume: 0.5mL DMSO
Compounds:
1. Amitriptyline
2. Methacycline
3. Tripolidine

Same Performance on 4.6, 21.2 and 30mm Diameter Columns

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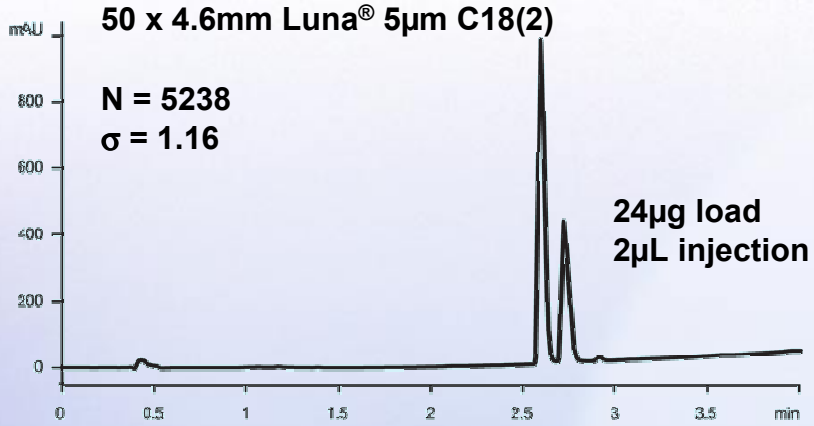
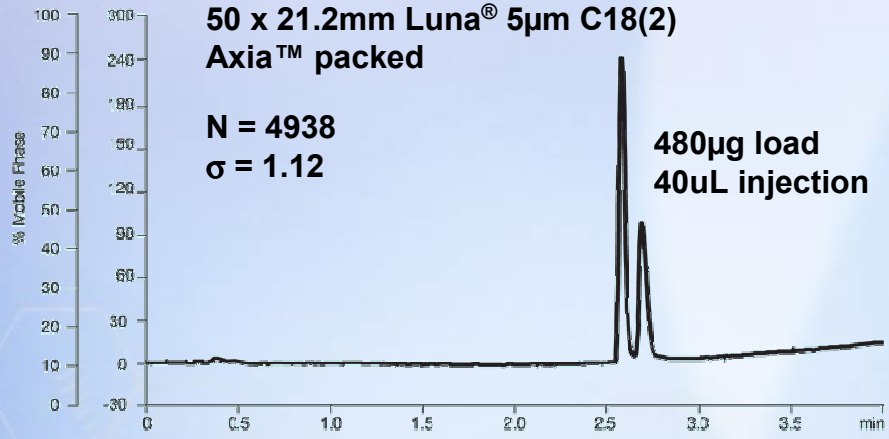
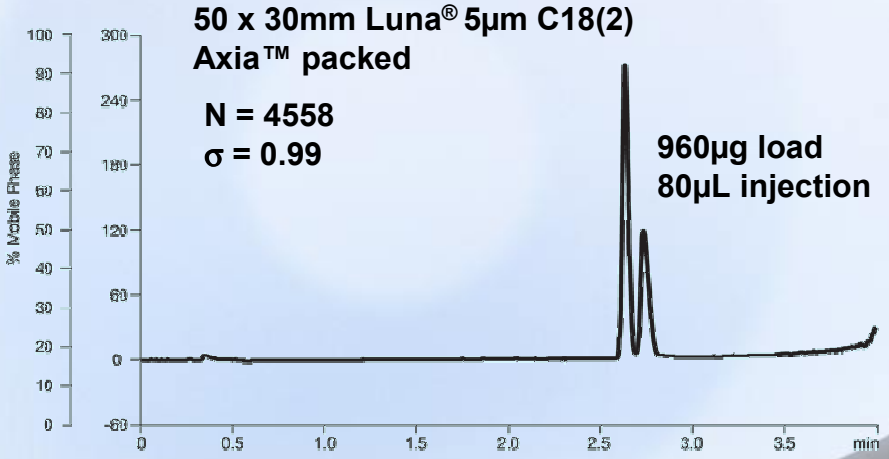


Gradient: 4 min 5% to 95% Water/Acetonitrile, 0.5% TFA
Flow rate: 1.5mL/min on 4.6,
30mL/min on 21.2
and 60mL/min on 30mm column
Detection: UV @ 254nm
Compounds: 1) Propranolol
2) Diphenhydramine





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Available HPLC phases

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explore LUNA®

C18(2), C8(2), Phenyl-Hexyl,

NH2, Silica 5µ & 10µ

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Max-RP, Hydro-RP,

Polar-RP, Fusion-RP 4µ & 10µ

Gemini™

C18, C6-Phenyl 5µ & 10µ

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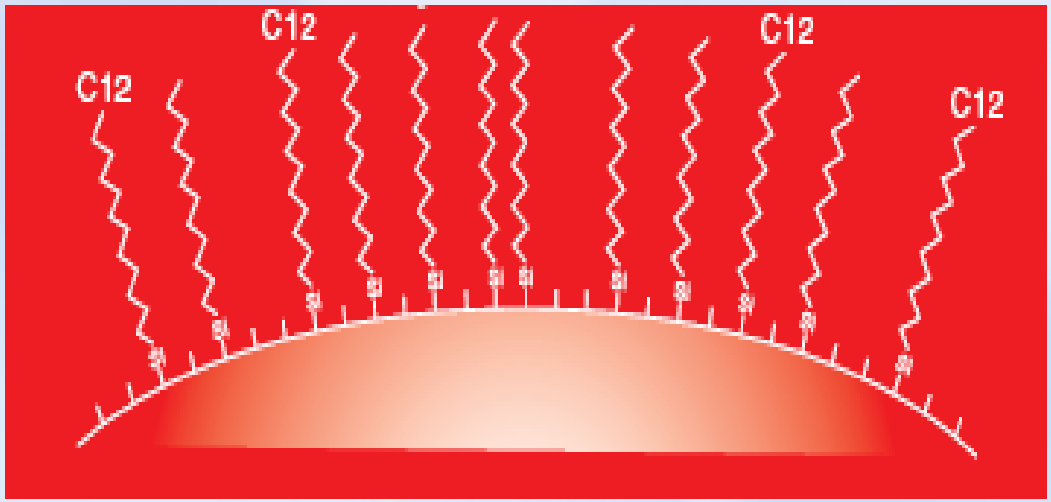
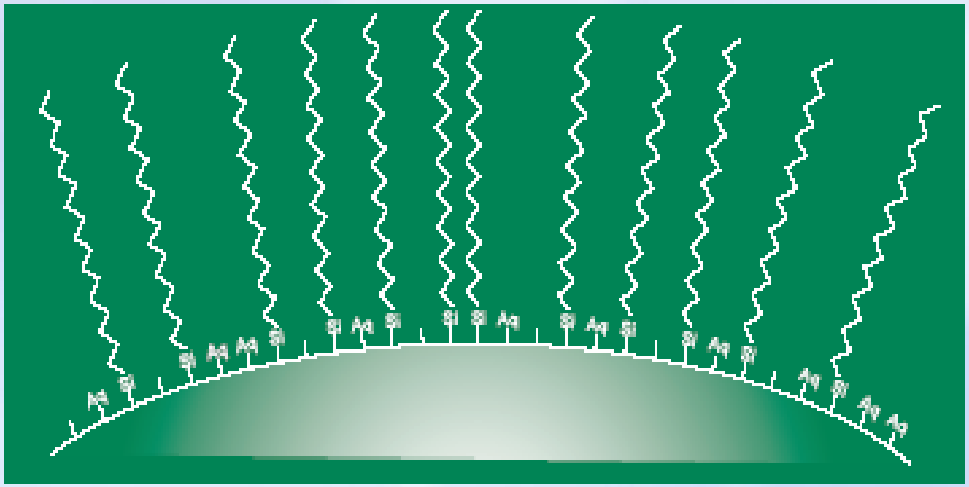
Synergi® Max-RP

- C12 phase
- 2.5 µ, 4 µ and 10 µ
- pH-Stability between pH 1.5 and pH 10
- Excellent for basic compounds



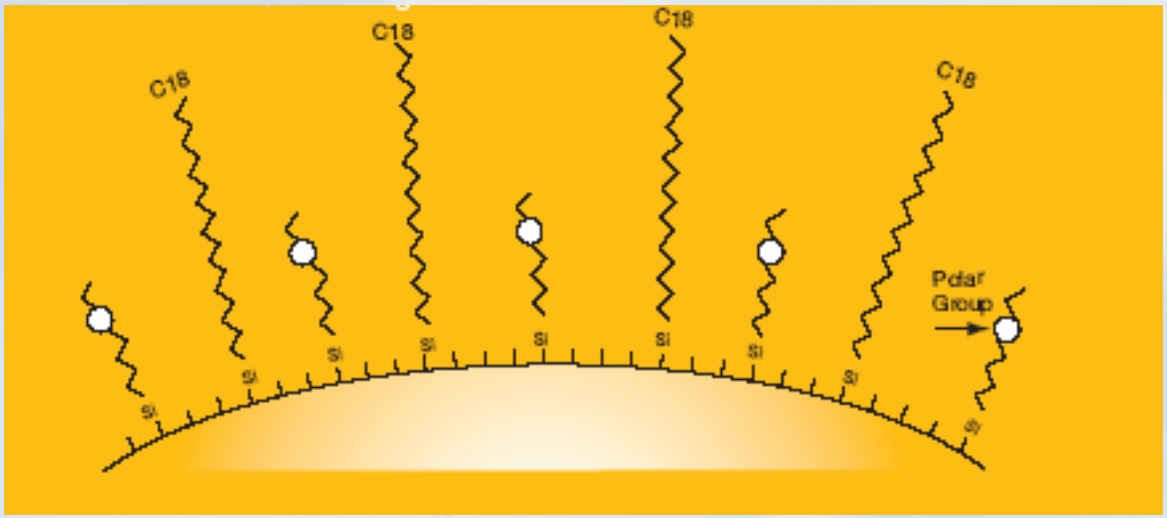
Synergi® Hydro-RP

- Hydrophobic C18 phase
- 2.5 µ, 4 µ and 10 µ
- 100% aqueous stable
- High methylene selectivity



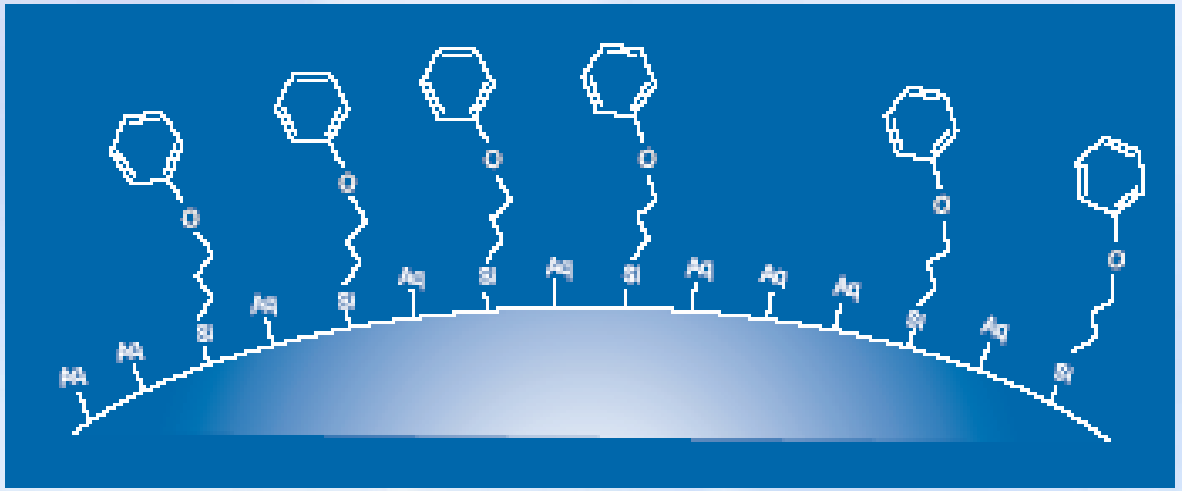
Synergi® Fusion-RP

- Polar embedded phase
- 2.5 µ, 4 µ and 10 µ
- pH Stability between pH 1.5 and pH 10
- 100% aqueous stable
- Extreme low bleed for LC/MS



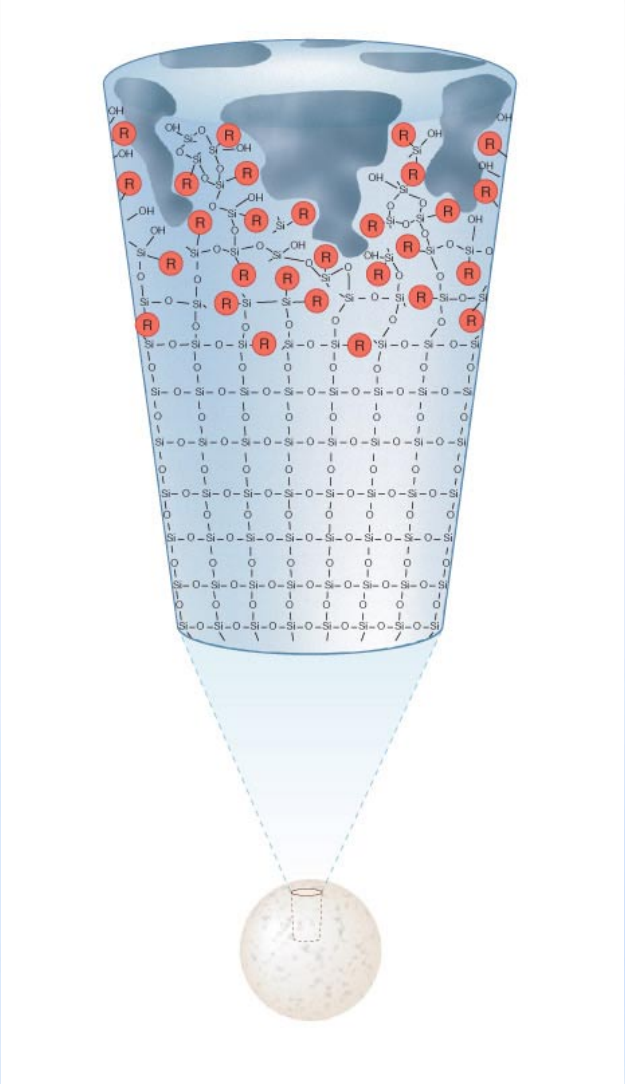
Synergi® Polar-RP

- Polar Phenyl phase
- 2.5 µ, 4 µ and 10 µ
- 100% aqueous stable
- Excellent for polar aromatic compounds



Gemini®

- Twin™-Particle
- Performance & Mechanical Strength of Silica
- pH-stable 1-12
- 3µ, 5µ, 10µ



Preparative Purification at High pH with Gemini®

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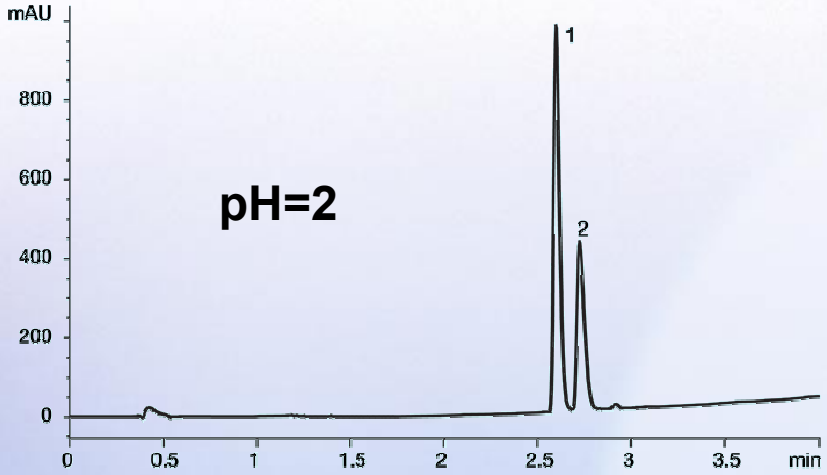
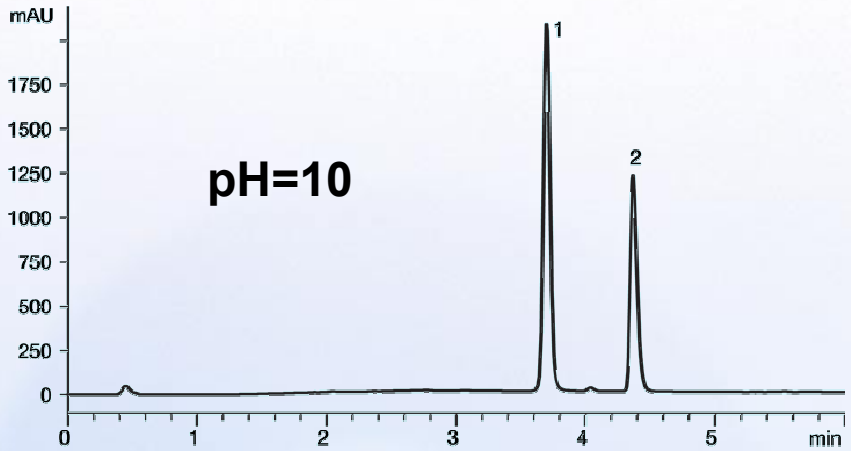


Column: Luna® 5µm C18(2) 50 x 21.2mm Axia™ Packed
Gradient: 4 min 5% to 95% Acetonitrile, 0.5% TFA
Flow rate: 30mL/min
Detection: UV @ 254nm
Compounds: 1. Propranolol
2. Diphenhydramine



Column: Gemini® 5µm C18 50 x 21.2mm Axia™ Packed
Gradient: 5 min 95:5 to 5:95 50mM NH_4HCO_3 pH10.0 /Acetonitrile
Flow rate: 30mL/min
Detection: UV @ 254 nm
Compounds: 1. Propranolol
2. Diphenhydramine

High pH dramatically improves resolution

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Advantages of Preparative Purification at High pH with Gemini®

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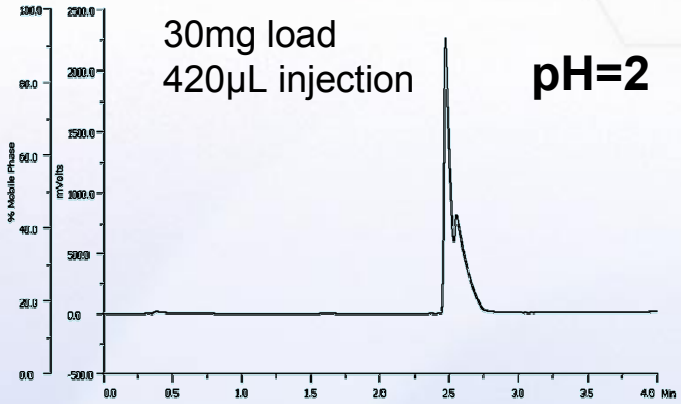
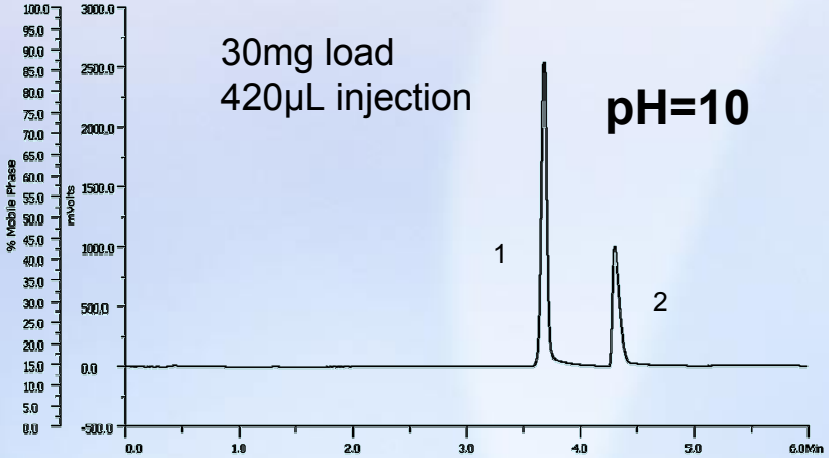
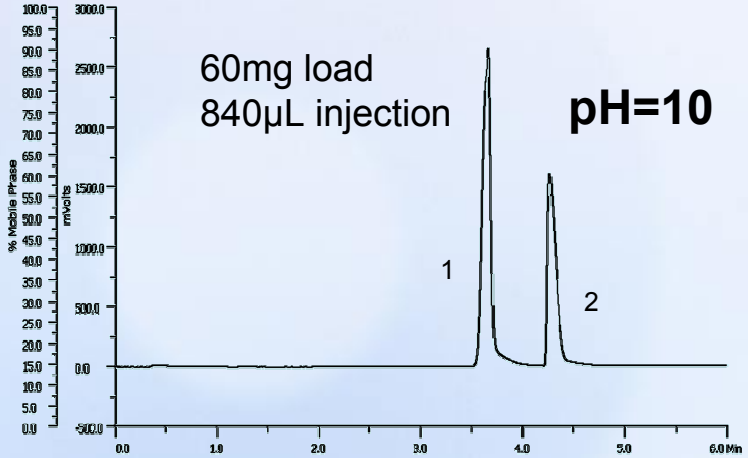
50 x 21.2mm Luna® 5µm C18(2)
(Axia™ Packed)







- Increased resolution between propranolol (1) and diphenhydramine (2)
- Increases preparative loading levels & Yields higher purity fractions

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Summary of Axia™ Technology

- **Eliminates bed collapse failure in high speed, high throughput preparative columns**
  - No decompression or relaxation during manufacturing process
  - No manual manipulation/disruption of bed
  - No crushing of media during packing
  - Achieve proper packing density and uniformity
- **Automated and highly process controlled system**
  - Infinitely tunable for specific media
  - Monitor column formation during process
  - Software controlled packing process
- **Higher performance and more reproducible columns**
  - Improved average asymmetry and efficiency
  - New industry standard for consistency in preparative columns
  - Same performance for 4.6, 21.2 and 30mm i.d. columns

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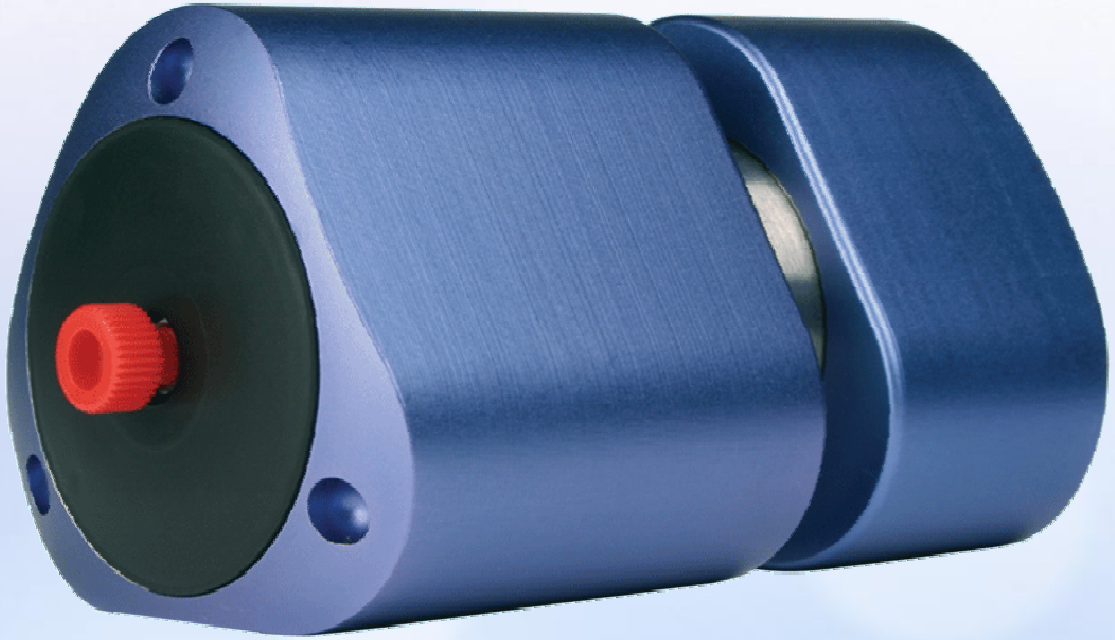
Questions?



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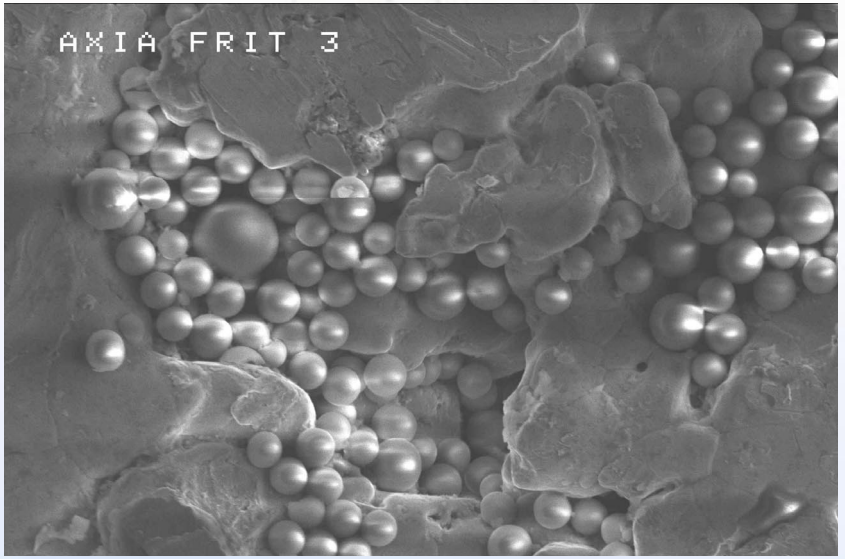
Axia™ Tuned Packing Process Eliminates Crushed Media

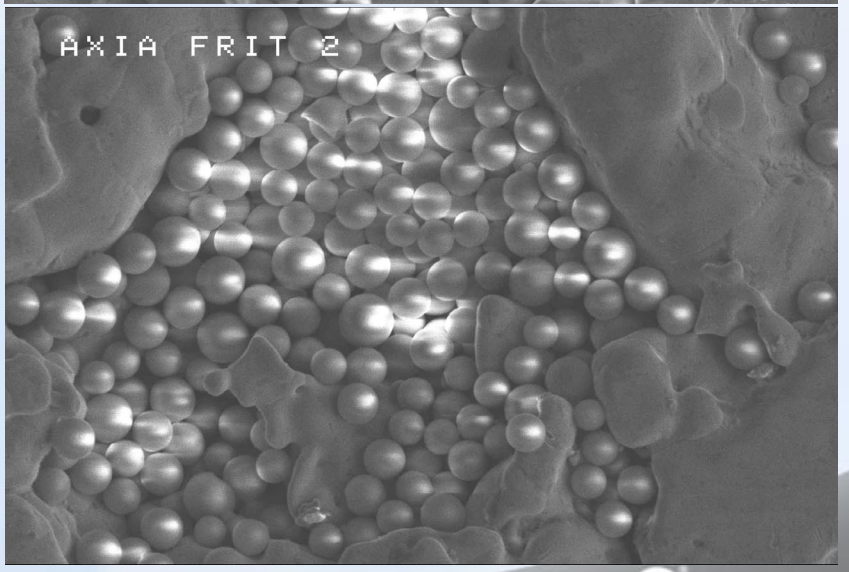
True optimization of bed density

- Infinite tuning of packing density

- Specific to media characteristics
  - Mechanical strength
  - Porosity

- Optimum chromatographic performance
  - Chromatographic efficiency
  - Peak asymmetry





SEM of Axia™ Frits after packing 50 x 21.2mm Luna® C18(2) 5µm

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