Method Optimization for Packing Various Polymer and Silica Chromatographic Medias with Dynamic Axial Compression Columns

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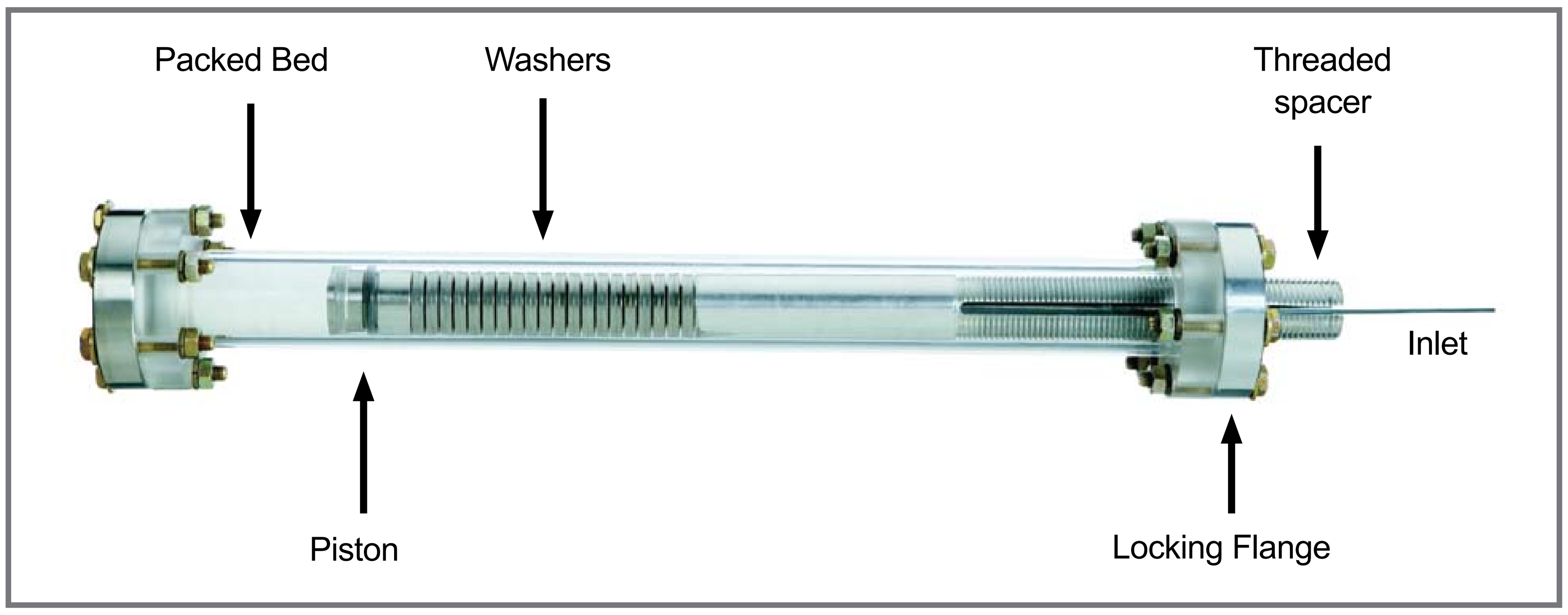
Introduction

One of the challenges in scaling up chromatography processes from analytical method development to pilot or production scale is optimizing the column packing procedure. Early method development work is typically done on pre-packed columns supplied from HPLC stationary phase manufacturers. However, for performance, economic, availability, and simple logistics issues most users migrate to dynamic axial compression (DAC) columns that are packed in-house once processes require large i.d. columns, >100mm i.d. Chromatographers experience numerous difficulties packing stable beds in process/production columns. These problems include non-uniform compression, bed deformation, channeling, void formation and crushing under improper packing. The use of true dynamic axial compression can overcome these issues and produce high quality beds that will exhibit high performance over a broad range of flow rates as evidenced by HETP (height per each theoretical plate) test. Placing constant pressure on a moveable piston gives it the dynamic capability of maintaining a consistent homogeneous bed. The resulting elimination of voids and channeling provides ultimate chromatographic performance. While stationary phase manufacturers may give guidance on packing procedures for their phases, end users often must engage in a trial and error process to determine how best to pack an HPLC phase using their individual DAC system, often sacrificing valuable product in the process. Here we outline a method for optimizing DAC packing using the MODcol® MultiPacker® columns for various HPLC phases including polymer resins, 300Å silica phases, 120Å silica phases, and irregular 60Å and 500Å silica. The availability of smaller i.d. DAC columns with the MODcol® system helps allow users to lock-in scaleable packing processes early in development to further minimize risk involved in scale-up. The Spring® column's main feature is the presence of a set of Belleville washers which provides constant pressure on the resin bed. Examples are given for DAC columns ranging from 25mm i.d. to 101mm i.d.

MultiPacker® Instrument and Spring® Column Overview

All columns were packed using the MODcol® MultiPacker® instrument and Spring® column. An illustration of the components of the Spring® column are below (**Figure 1**).

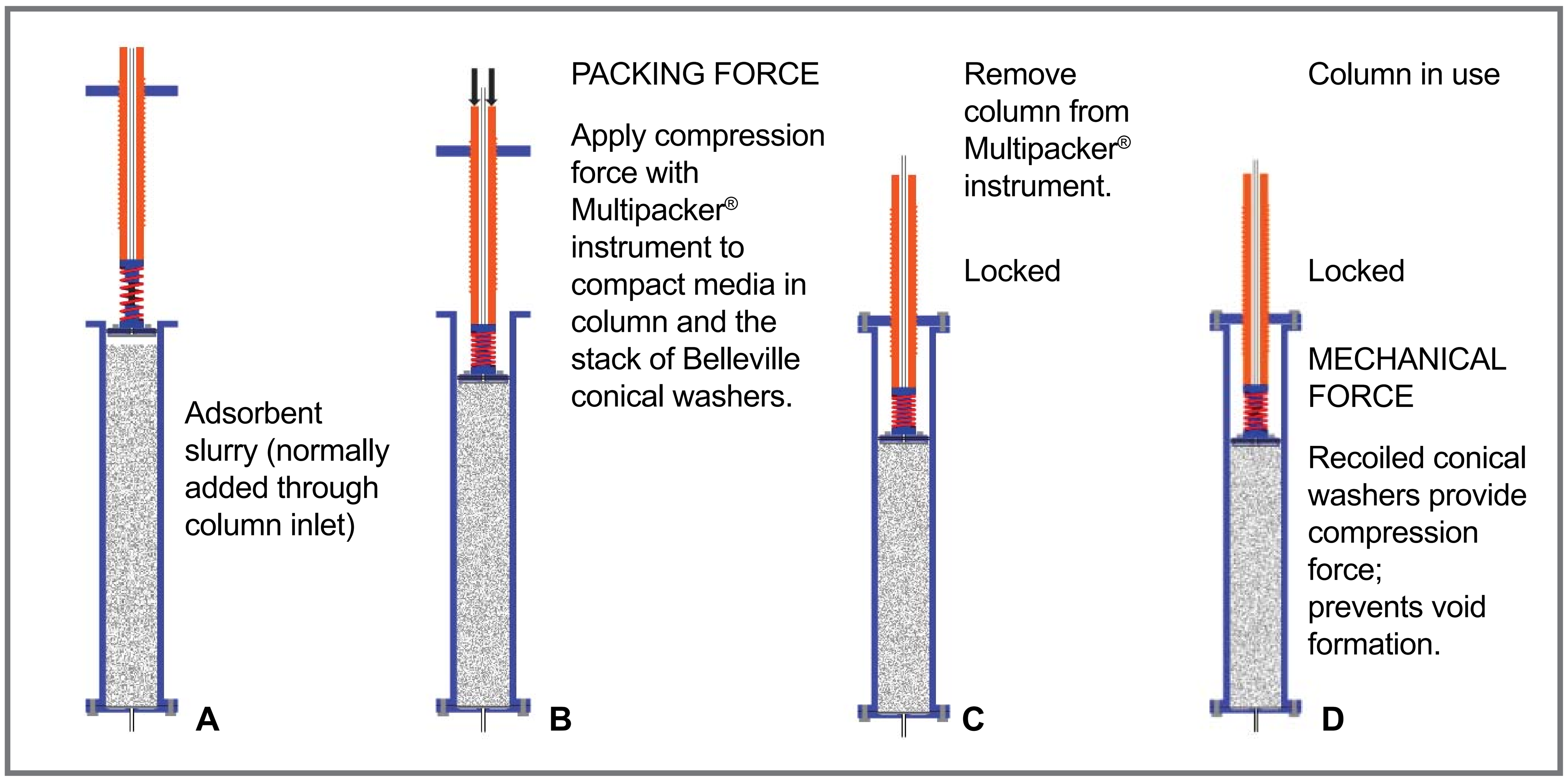
Figure 1. Spring® Column Design



Spring® columns are packed using a MultiPacker® instrument (**Figure 2**) which is specifically designed for use with the Spring® column. The MultiPacker® instrument packing station provides the ability to control packing pressure independent of packing speed. **Figure 3** provides an overview of the general packing procedure.

Figure 2. MODcol® MultiPacker® Instruments

Figure 3. Packing Steps



Advantages of MODcol® Spring® Column Technology

- Self contained prepacked axial compression column
- High Column Efficiency
- Repeatability (**Figure 4**)
- Portability (**Figure 5**)
- Cost of Ownership Is Less Than Prepacked Columns
- Improved Long-term Performance Versus Prepacked Columns
- Fault-tolerant Mechanical Design
- Rapid Packing, Unpacking, Cleaning and Repacking

Figure 4. Spring® Column Reproducibility (Media: Vydac® 15-20µm silica, 300Å, C18 218TPB1520)

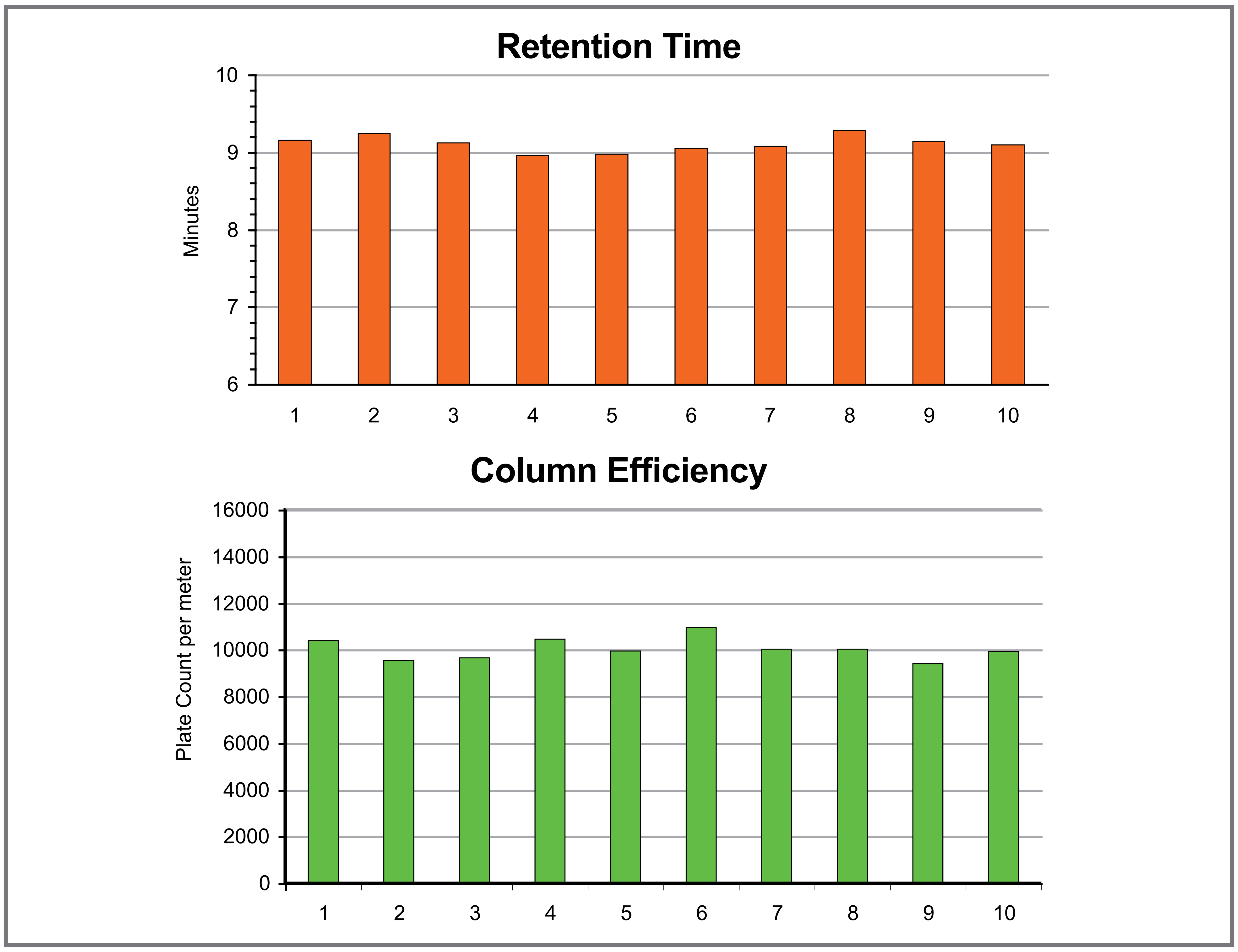


Figure 5. Spring® Column Portability

Spring® columns can be removed from the packing stations while maintaining dynamic axial compression due to the mechanical compression force.

Critical Success Factors in Dynamic Axial Compression Packing

Successful column packing is primarily impacted by slurry conditions and piston pressure. General guidelines were developed to assist in determining optimum packing conditions. These conditions are recommended based on our experiences using the MODcol® MultiPacker® instrument and may not hold for other DAC systems.

Slurry Conditions

- Effective DAC packing requires slurry conditions that sufficiently disperse the media (solvent polarity) while maintaining adequate solvent viscosity. Therefore, appropriate selection of an organic slurry solvent, or combination of solvents, is critical. General guidelines for choosing slurry solvents can be found below:
  - The type of stationery phase dictates the general polarity of slurry solvent being used.

Phase:	Reversed	Normal	Chiral	Ion Exchange
Solvent:	Polar (high-low)	Polar (low)	Polar (low)	Polar (high)

  - For reversed phase, the relative polar strength of the solvent can be based on %C (hydrophobicity) of the stationary phase.

% Carbon:	High (C18)	Low (C4)
Relative Polarity:	High	Low

  - Below are a few slurry solvents that were found to give good packing results in each of these categories.

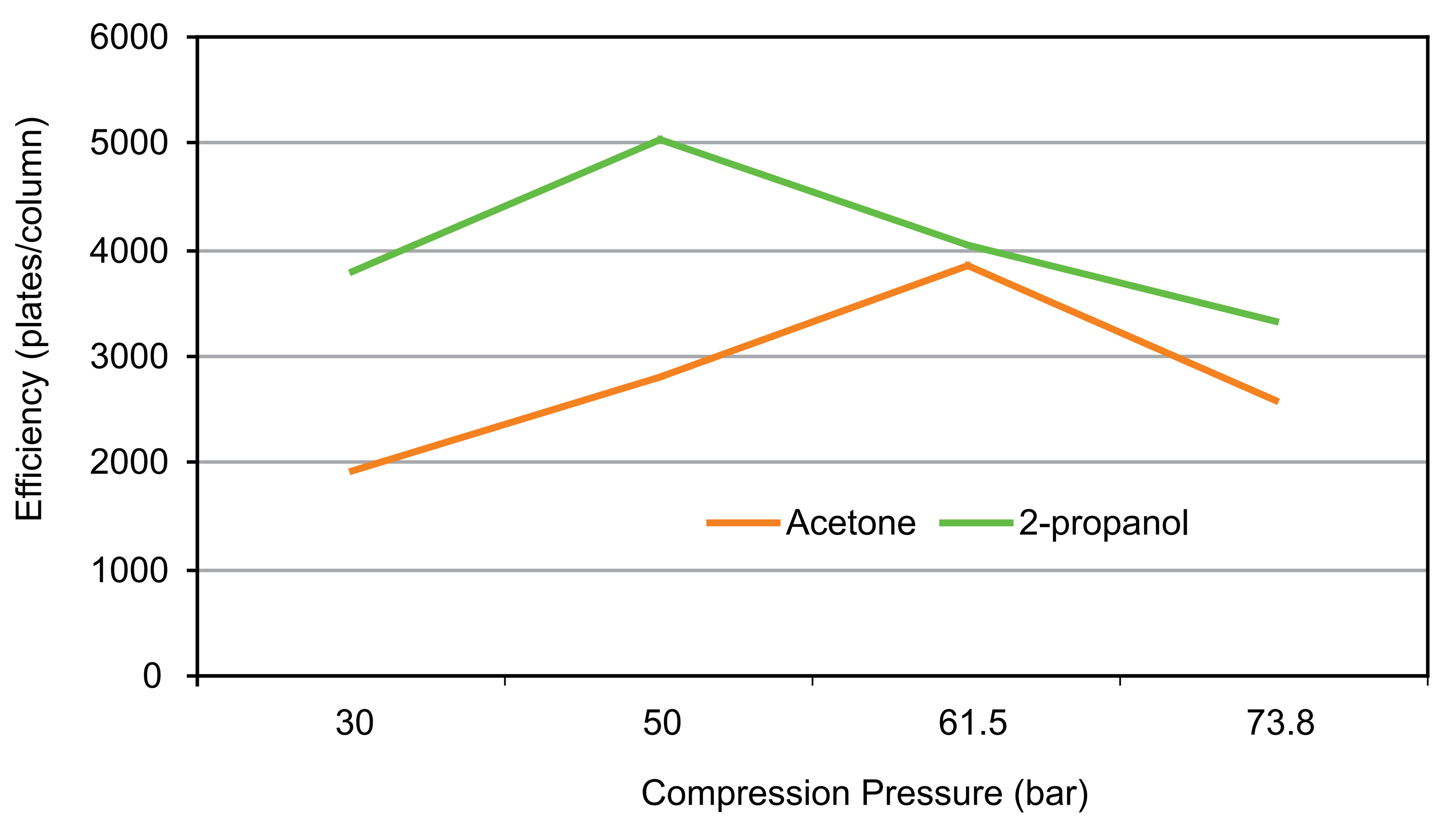
Solvent Examples	
Polar (high)	**Polar (low)**
Methanol	Ethanol
Acetone	2-Propanol
2-Propanol:Water	
Ethanol:Water	

  - The viscosity of the slurry solvent plays an important role in dictating the speed at which the column is packed. Typically, a slurry solvent with a high viscosity works better than a low viscosity. A viscosity approaching 2-propanol (2.4 centipoise @ 20°C) works well for most stationary phases.

Piston Pressure

- Packing pressure is a critical variable in packing a high efficiency column. However, in addition to media characteristics impacting the optimum packing pressure, slurry solvent can also play a role (**Figure 6**). This further complicates the selection of the appropriate packing pressure.

Figure 6. Effect of Compression Pressure and Slurry Solvent on Column Efficiency for Vydac® 300Å, C4, 20-30µm Media



- Particle size

Particle Size:	Small	Large
Packing Pressure:	High	Low

- Pore Volume/Pore Diameter

Pore Diameter:	Small	Large
Packing Pressure:	High	Low

- Particle Shape

Particle Shape:	Spherical	Irregular
Packing Pressure:	High	Low

Experimental

- MODcol® Spring® columns were packed with
  - Vydac® TP 300Å C4 silica
  - Polymer Labs PLRP-S 100Å 10µm media
  - Vydac® Denali® 120Å C18 silica
  - Chiralcel® OD media
  - Davisil® XWP500Å 35-70µm silica
  - Davisil® Diol Bonded 60Å 9.5-11µm silica
  - Davisil® Diol Bonded 60Å 40-63µm silica

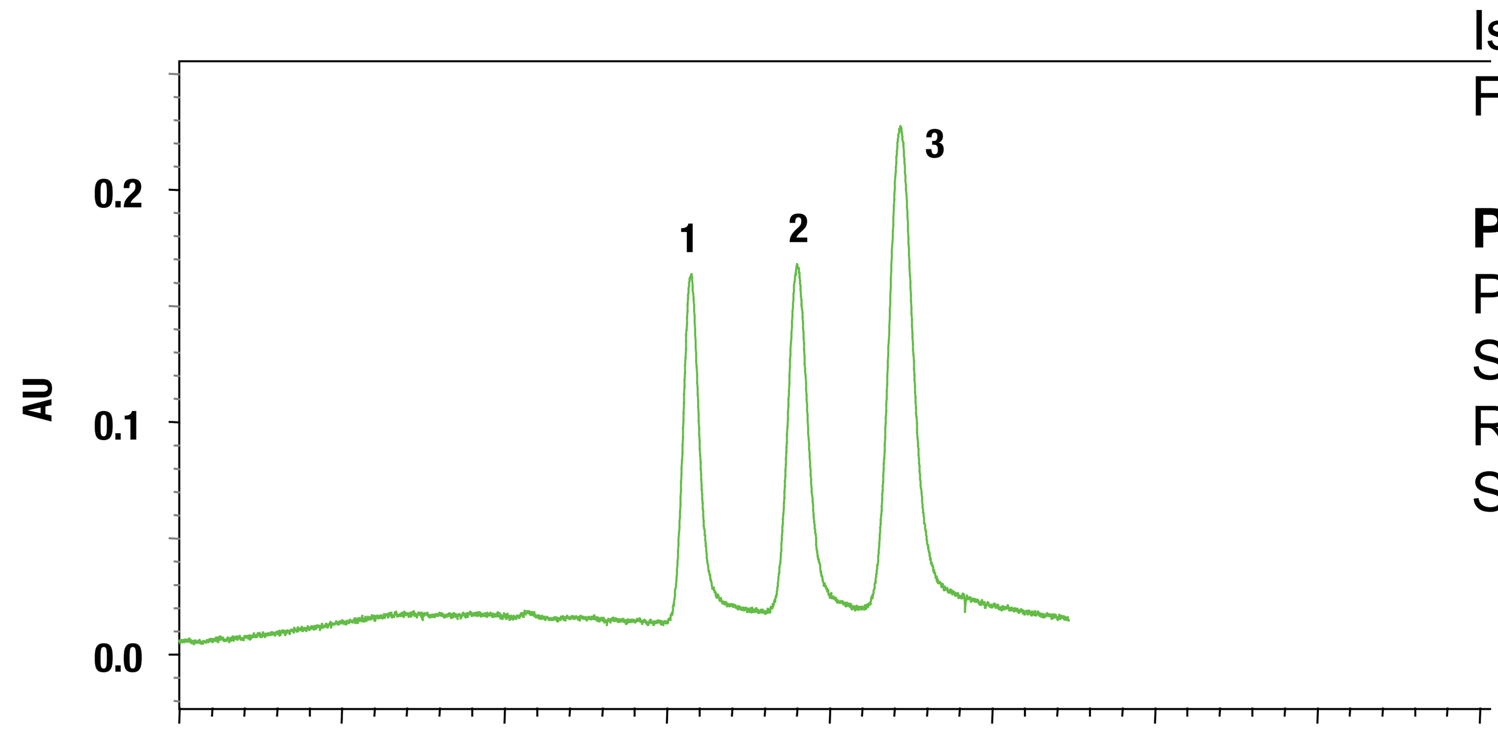
Efficiency tests were performed and optimized packing conditions noted to provide guidance for packing these and similar stationary phases.

- Vydac® TP 300Å silica was packed in larger Spring® column i.d.s (101mm) to test the scalability of the MODcol® Spring® columns, especially at larger column i.d. with a wide pore silica.
- Efficiency tests were performed on the Polymer Labs PLRP-S 100Å resin after equilibrating with 10, 600, and 1000 column volumes to gauge the suitability of the Spring® column for packing polymer based material.

Results

Vydac® Media C4, 300Å, 20-30µm

Column Dimension: 101mm i.d.x 300mm length



Run Conditions:
Isocratic 50:50 Acetonitrile:Water
Flow Rate: 200mL/min.

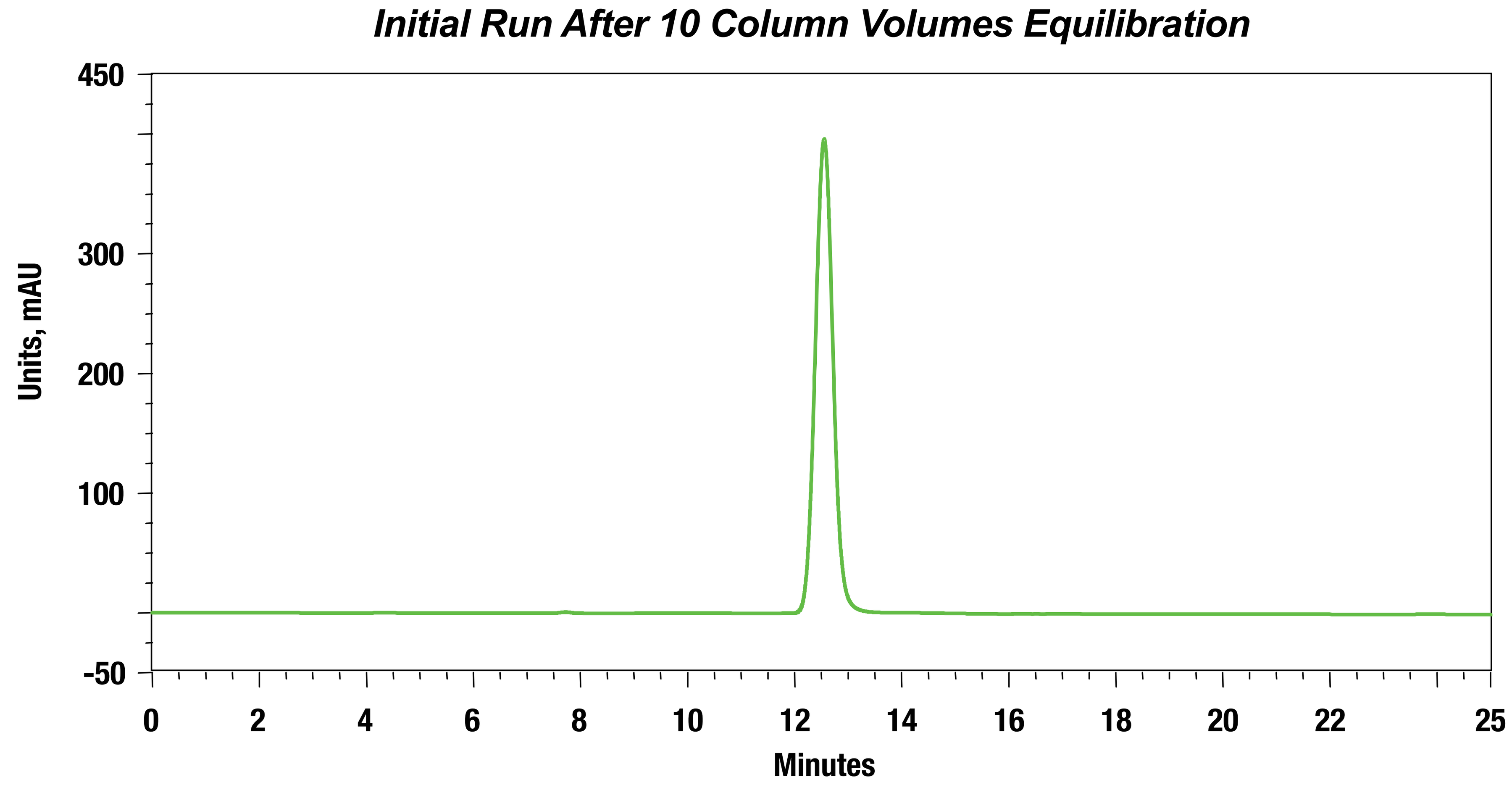
Packing Conditions:
Packing Pressure: 800 psig
Slurry Solvent: 100% 2-Propanol
Resin Amount: 1220 grams
Slurry Solvent Volume: 5000mL

Peak No.	Name	Retention Time	Plates/column	Plates/meter	Asymmetry (10%)
1	Benzene	15.75	4995	16650	1.06
2	Naphthalene	19.00	4893	16310	1.08
3	Biphenyl	22.18	4249	14160	1.11

High efficiency is obtained even at low packing pressures needed for wide pore silica

Polymer Reversed Phase PLRP-S 100Å 10µm media

Spring® Column: 25mm i.d. x 150mm length

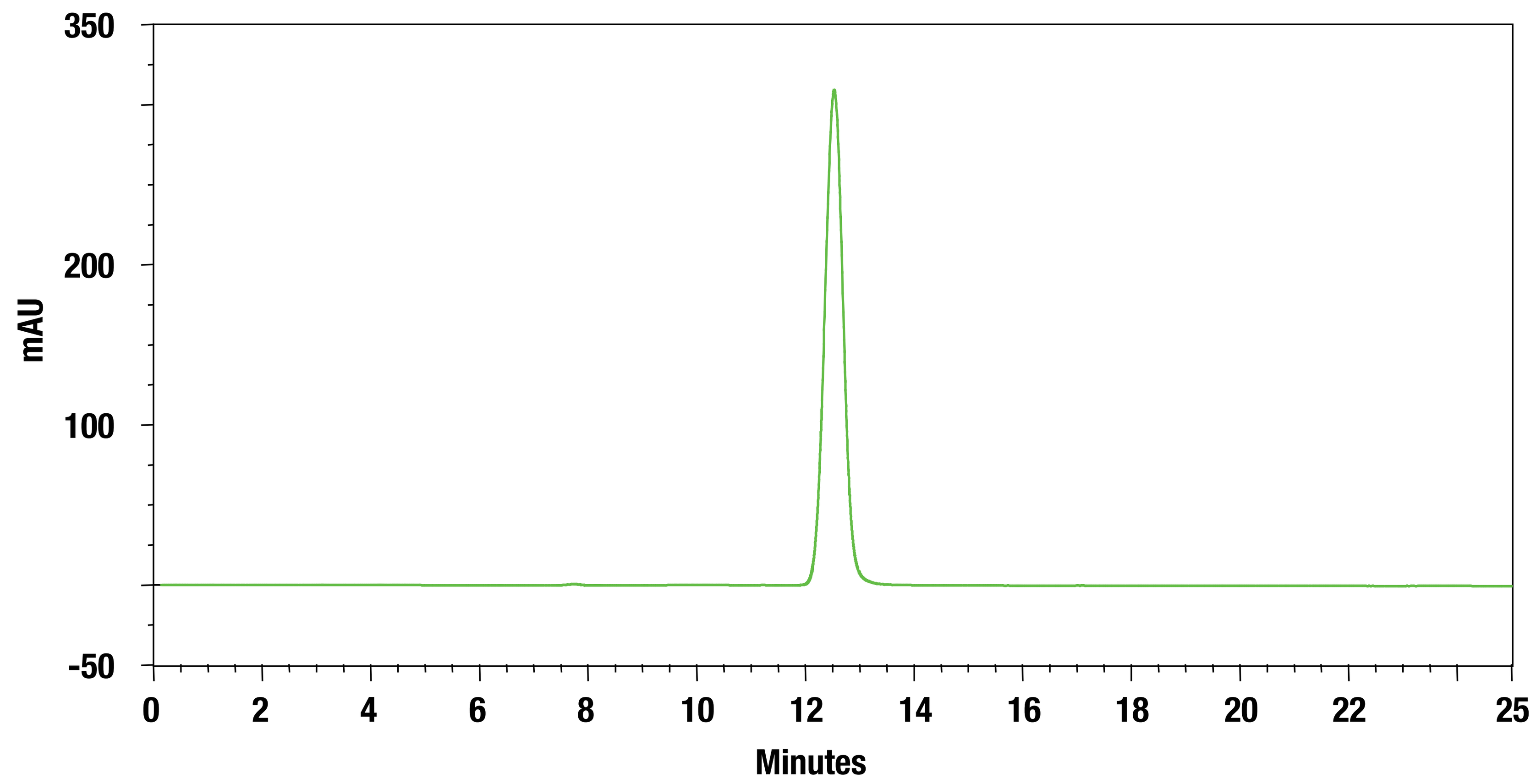


Run Conditions:
Isocratic: 70:30 Acetonitrile:Water
Flow Rate: 10mL/min.
Concentration: 4mg/mL
Injection Volume: 20µl
Analyte: Phenol
Detection: 254nm

Packing Conditions:
Packing Pressure: 800 psig
Slurry Solvent: 100% 2-Propanol
Resin Amount: 40.5 grams
Slurry Solvent Volume: 160mL

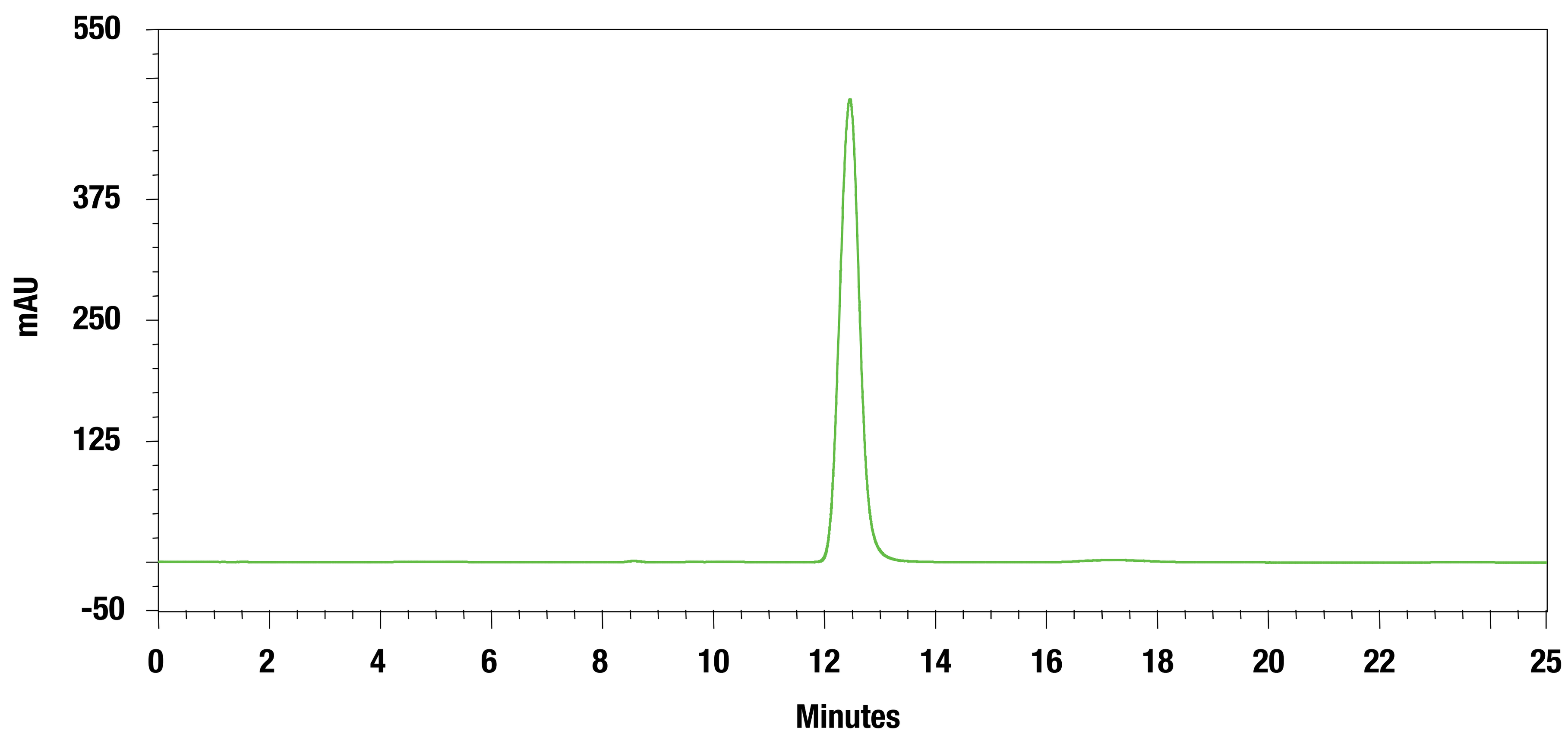
Retention Time	Efficiency Plates per Meter	Asymmetry
12.544	45500	1.03

Run After 600 Column Volumes Equilibration



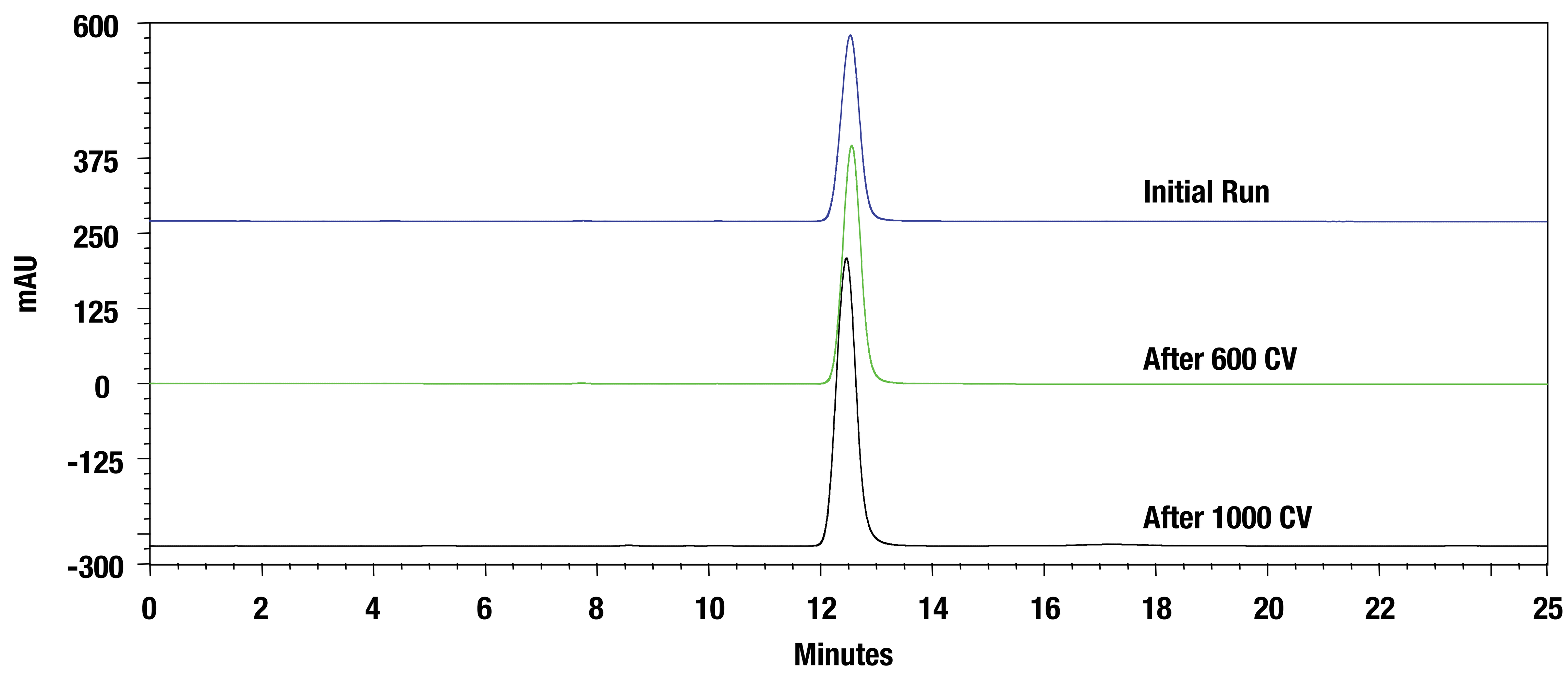
Retention Time	Efficiency Plates per Meter	Asymmetry
12.537	42667	0.99

Run After 1000 Column Volumes Equilibration



Retention Time	Efficiency Plates per Meter	Asymmetry
12.462	37807	1.03

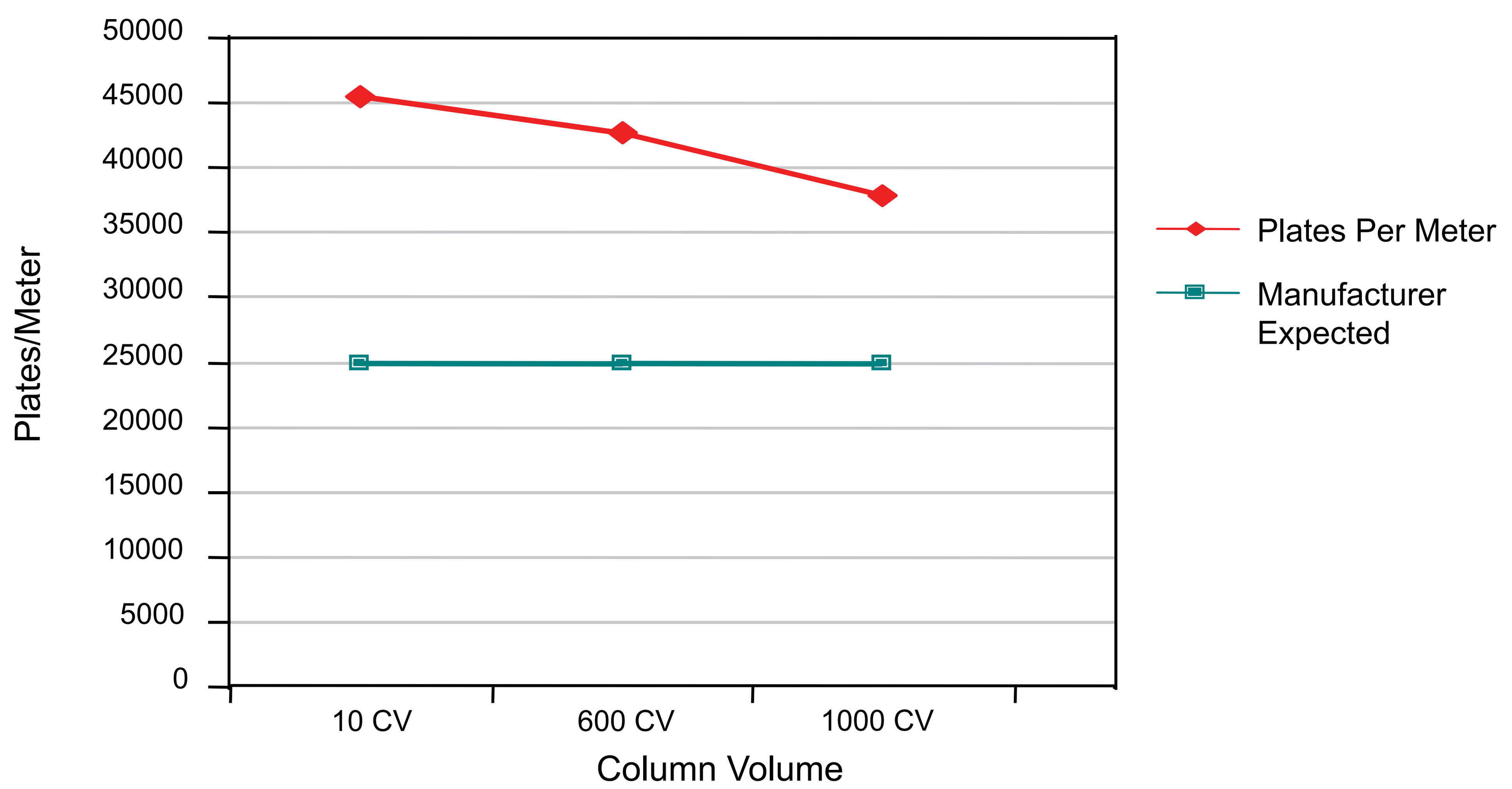
After 1000 (74,000mL) Column Volumes



Column Volume	Retention Time	Asymmetry	Plates Per Meter
10	12.544	1.03	45500
600	12.537	0.99	42667
1000	12.462	1.03	37807

Retention time is unchanged and excellent asymmetry is observed from start to finish

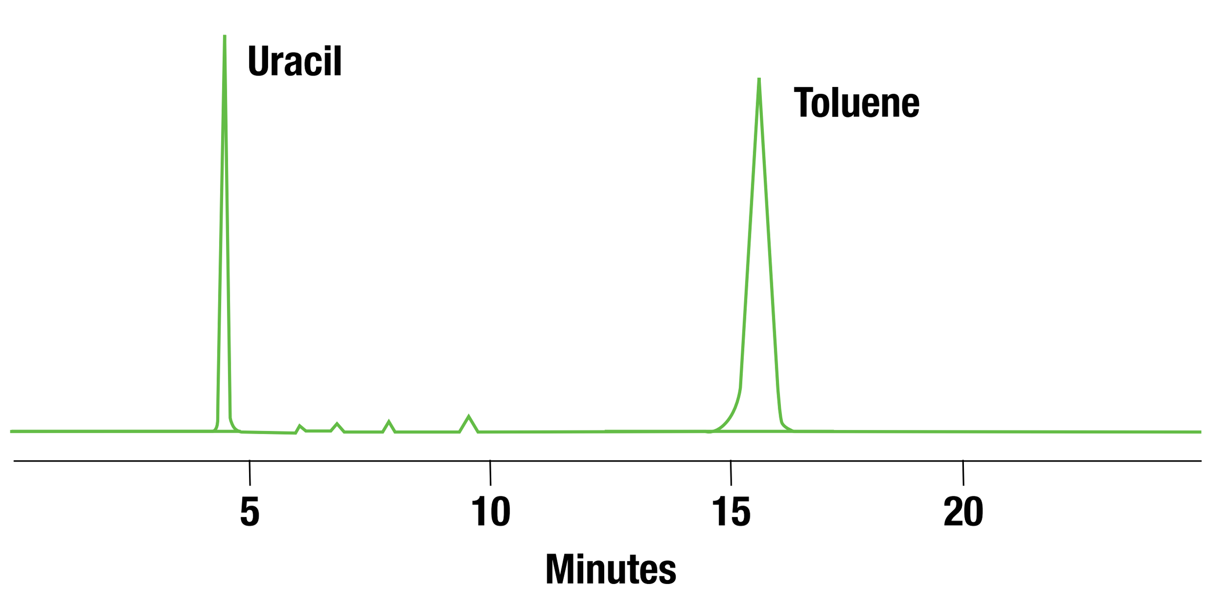
Column Performance Results After 1000 Column Volumes



Higher than expected efficiency is maintained over 1000CV

Vydac® Media Denali® C18, 120Å, 10µm

Spring® Column: 50mm i.d.x 250mm length



Run Conditions:
Isocratic: 70/30 AcN/H_2O
Flow Rate: 60mL/min.

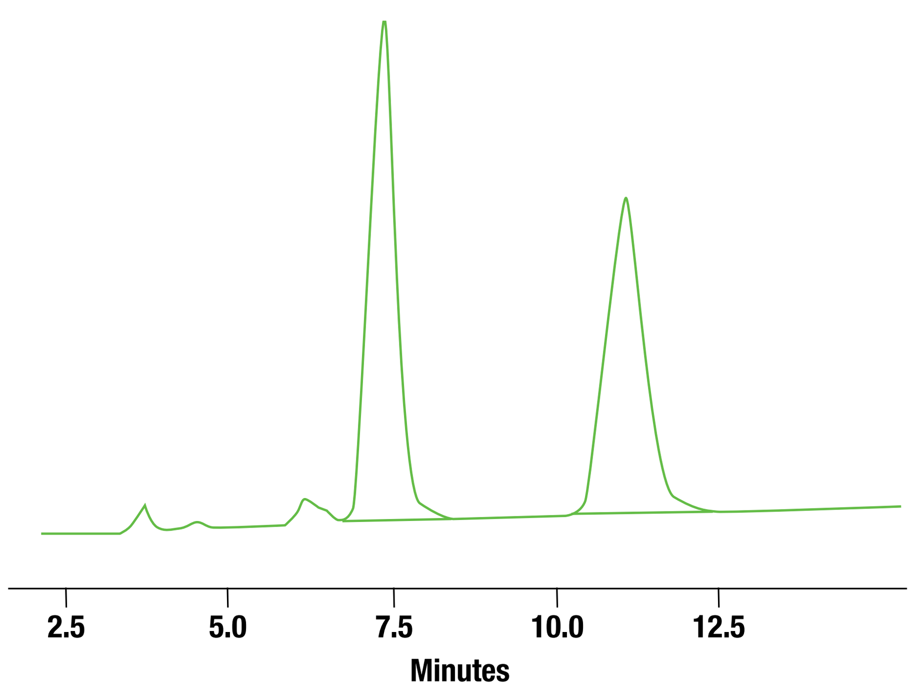
Packing Conditions:
Packing Pressure: 2000 psig
Slurry Solvent: 80% Chloroform 20% 2-Propanol
Resin Amount: 305 grams
Slurry Solvent Volume: 1200mL

Solute	Retention Time	Plates per Column	Asymmetry (10%)	Plates per Meter
Uracil	4.41	8121	1.00	32484
Toluene	15.68	7098	1.00	28391

A high density slurry solvent can be used for packing reversed-phase media with superb peak symmetry.

Diacel Chiralcel® OD 20µm media

Spring® Column: 50mm i.d. x 150mm length



Run Conditions:
Isocratic: 90/10 Hexane/2-propanol
Flow Rate: 60mL/min.

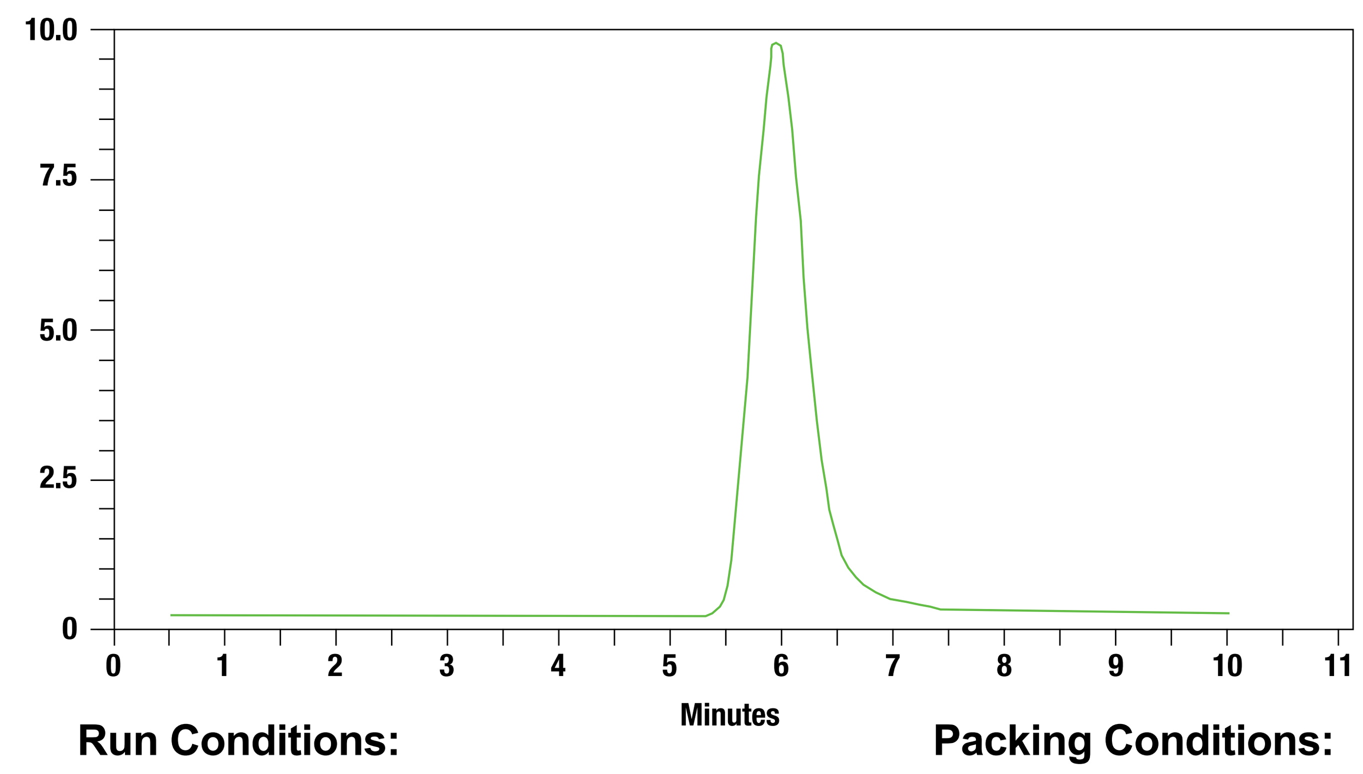
Packing Conditions:
Packing Pressure: 1000 psig
Slurry Solvent: 100% 2-Propanol
Resin Amount: 185 grams
Slurry Solvent Volume: 700mL

Solute	Retention Time (min.)	Plates per column	Asymmetry (10%)	Plates per meter
Peak 1	7.38	2100	1.02	13816
Peak 2	11.09	1832	1.06	12050

Medium pressure (1000 psig) plus low polarity slurry solvent provides the best packing conditions for Diacel material.

Davisil® XWP500Å 35-70µm silica

Spring® Column: 50mm i.d. x 230mm length



Run Conditions:
Isocratic: 90:10 Heptane:2-Propanol
Flow Rate: 85mL/min.

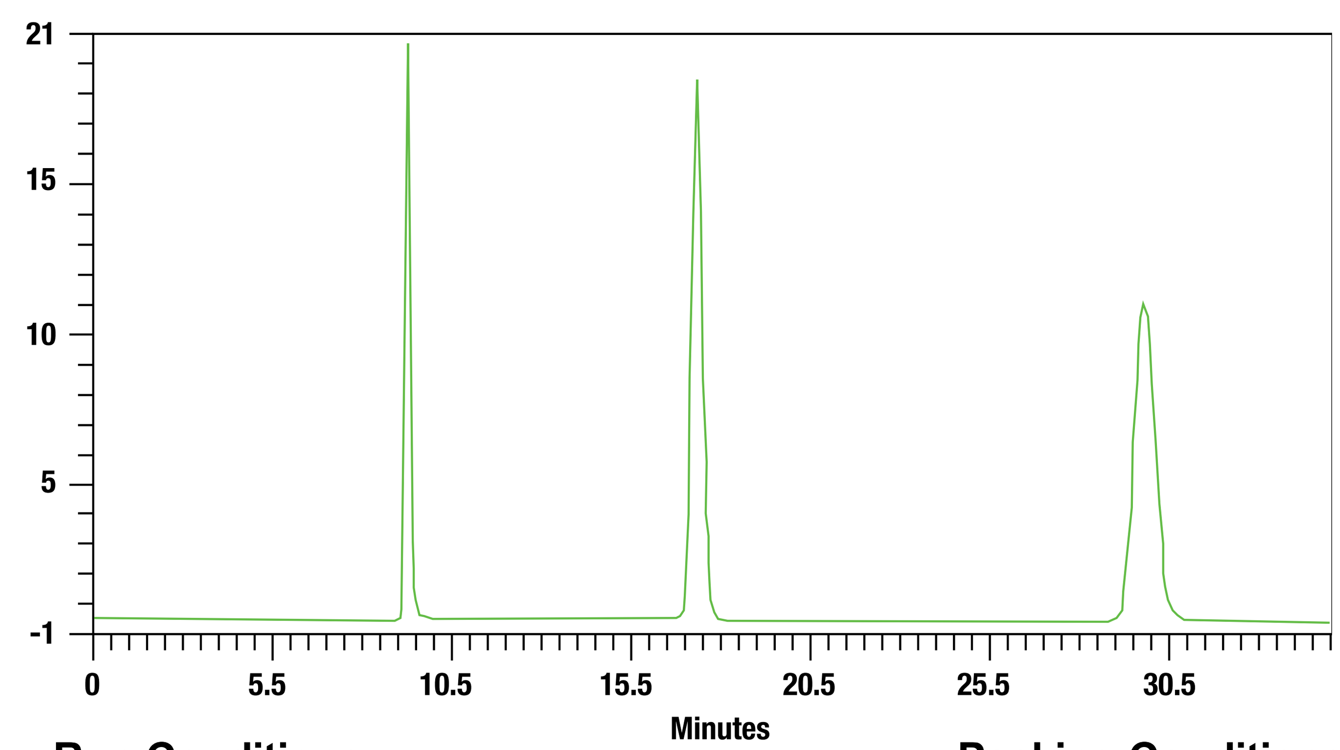
Packing Conditions:
Packing Pressure: 653 psig
Slurry Solvent: 100% 2-Propanol
Resin Amount: 165 grams
Slurry Solvent Volume: 650mL
Packing Speed: Slow, approx. 1mm/sec

Solute	Retention Time	Plates per Column	Plates per Meter	Asymmetry (10%)
Dimethylphthalate	5.94	615	2674	1.479

Extra Wide Pore (XWP), irregular silica can be packed successfully under axial compression conditions.

Davisil® 710N2OH 60Å 10-14µm silica

Spring® Column: 101mm i.d. x 340mm length



Run Conditions:
Isocratic: 90:10 Heptane:2-Propanol
Flow Rate: 250mL/min.

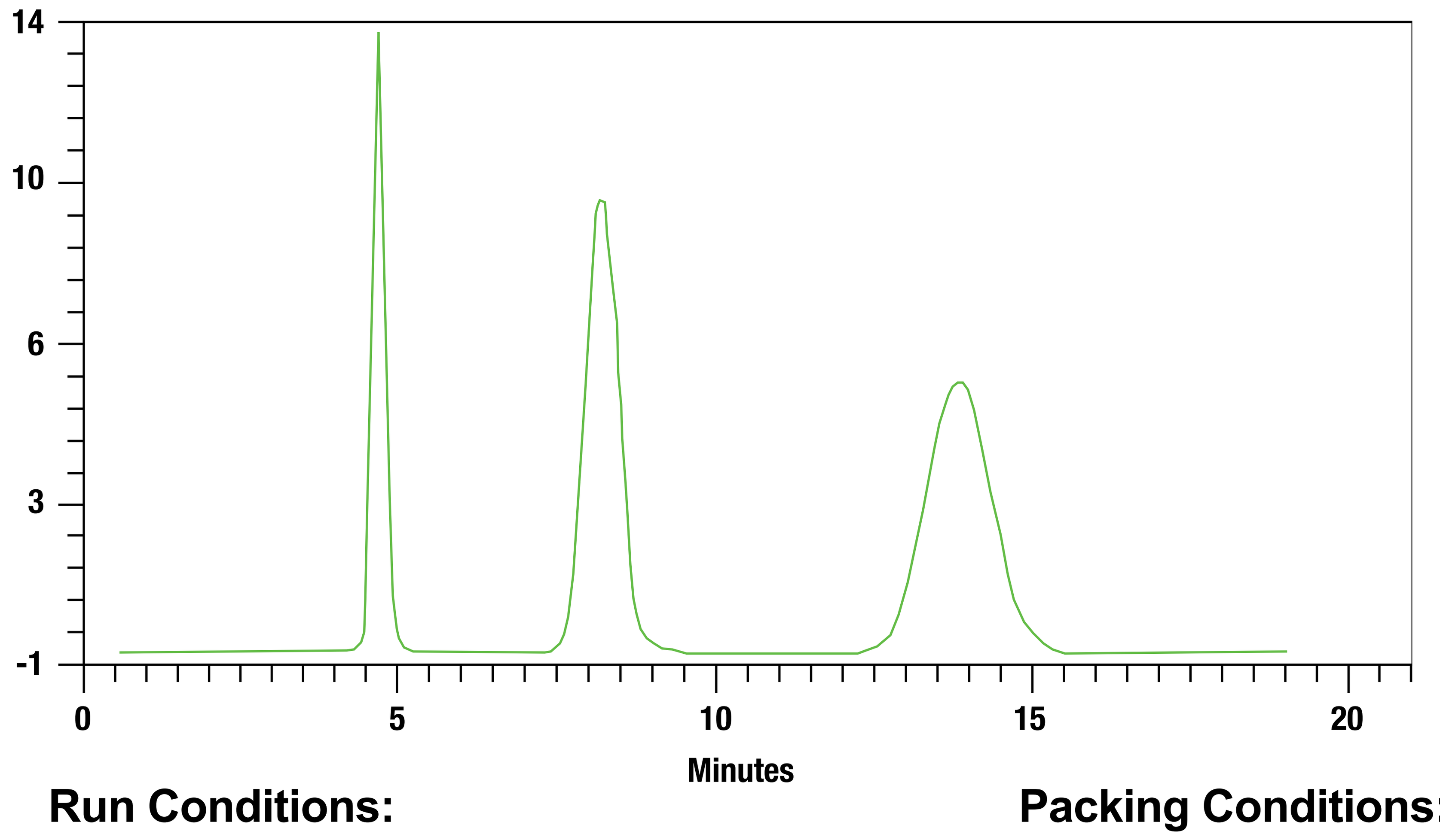
Packing Conditions:
Packing Pressure: 623 psig
Slurry Solvent: 100% 2-Propanol
Resin Amount: 1315 grams
Slurry Solvent Volume: 4000mL
Packing Speed: 0.2cm/sec

Peak No.	Name	Retention Time	Plates per Column	Plates per Meter	Asymmetry (10%)
1	Toluene	9.33	15576	45812	1.250
2	2-Chlorophenol	17.38	14212	41800	1.152
3	4-Nitrophenol	29.81	11569	34026	1.119

Diol bonded irregular silica can be packed successfully under axial compression conditions.

Davisil® 633N2OH 60Å 47-60µm silica

Spring® Column: 101mm i.d. x 142mm length



Run Conditions:
Isocratic: 90:10 Heptane:2-Propanol
Flow Rate: 250mL/min.

Packing Conditions:
Packing Pressure: 435 psig
Slurry Solvent: 100% Ethanol
Resin Amount: 600 grams
Slurry Solvent Volume: 2000mL
Packing Speed: 0.5 - 1cm/sec

Peak No.	Name	Retention Time	Plates per Column	Plates per Meter	Asymmetry (10%)
1	Toluene	4.66	1761	12401	1.278
2	2-Chlorophenol	8.18	960	6761	1.168
3	4-Nitrophenol	13.79	720	5070	1.205

Diol bonded irregular silica can be packed successfully under axial compression conditions.

Conclusion

Successful packing using dynamic axial compression is impacted primarily by the slurry solvent and piston pressure. Numerous factors relating to the media choice dictate the optimum solvent and pressure conditions. General guidelines have been developed around these factors to help optimize packing conditions; however, because of the interrelationship of many factors, some experimentation is often necessary before the ideal conditions are determined. Therefore, it is important to begin the column packing process early in development to avoid costly packing issues at a large scale. The MODcol® MultiPacker® instruments and Spring® column offer a robust scaleable system that provides control of the essential packing variables needed for successfully packing of many different types of media. The system mobility increases its ease of use in often limited lab space.