Enhancing High Throughput Preparative HPLC: Offline Regeneration of Identical Superficially Porous Particle Preparative Columns Utilizing The 1290 Infinity II Preparative LC System and Agilent InfinityLab Poroshell 120 Preparative LC Columns to extend Column Lifetime, Minimize Sample Carryover and Increase Throughput

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Abstract

High throughput sample purification and analysis are important tasks in many industries. With the increasing number of samples synthesized– HPLC purification should not be the bottleneck in discovery and development. The throughput on the Prep system can be increased by optimizing the chromatographic method and by optimizing the instrument hardware. In this poster we show how the throughput can be enhanced even further by using two identical Poroshell Preparative HPLC columns in the system and operating them alternately optimizing the 1290 Infinity II Preparative LC System for Off-Line Column Regeneration. The greatest benefit of the offline column cleanup and equilibration is the increase in sample throughput, but added benefits include extended column lifetime and a reduction in sample carryover

Introduction

A normal Preparative HPLC run contains four steps: sample injection, gradient or isocratic elution run, column wash and column equilibration. The four steps of the preparative run are commonly performed sequentially and equal the total overall cycle time. In this example, the system has been optimized to improve throughput with the addition of a second and identical preparative column, high-pressure switching valve and an additional high flow binary preparative pump to take the last two steps from sequential to parallel processes. The second pump is used as a regeneration pump to both perform the column wash and equilibration on the column that is offline while the next analysis is running on the online column. For each sequential run, the columns are switched to alternate running and washing. This procedure is called alternating column regeneration. It is possible to maintain improved column efficiency over an extended period of time by ensuring that the column is properly washed, and impurities are not building up on the column. The build-up of sample impurities may cause loss of performance, an increase in back-pressure, peak tailing, retention time shift and/ or baseline drift. The use of the second pump to quickly ramp up flow on a poroshell column offline above the typical linear velocity for this column, saves time by cleaning the column more quickly, with lower backpressure and fast equilibration to ensure the column is not retaining impurities that can impair the performance of the column and that the column is in a steady equilibrated state for the next injection. Moving the cleaning and equilibration steps offline can shorten the total runtime substantially, often cutting the process in half.

Experimental

Hardware

- Agilent 1290 Infinity II Preparative Purification System
  - 2x G7161B
  - G7114A VWD
  - G7111B Quaternary Pump
  - G7115A Diode Array
  - 7158B Autosampler/Fraction Collector
- Agilent 1290 Infinity II Quaternary UHPLC

Software

- Agilent OpenLAB CSD ChemStation Edition C.01.08 [216]

Samples & Solvents

- Alll standards sourced from Sigma Aldrich
  - Acetylsalicylci Acid, Benzoic Acid, Phenol, Caffeine.
- All Solvents sourced from Agilent Technologies
  - Acetonitrile, MilliQ Water, Formic Acid

Columns.

- Agilent InfinityLab Poroshell 120,SB-C18,21.2x50mm,4um

Experimental

Analytical Method Development and Scaleup to Prep

The analytical method was developed on an Agilent Inf II UHPLC using a matching Poroshell SB C-18 column to the Preparative Poroshell SB C-18.(Fig 1) The loading study was focused on increasing load to maximize purification of the first peak in the mixture. The analytical method was scaled up from a 1ul injection to a 5ul, 10ul and 20ul loading study. The 20ul injection had breakthrough of the compound of interest, therefore the 10ul analytical method was scaled up for the preparative method. (Fig 2)

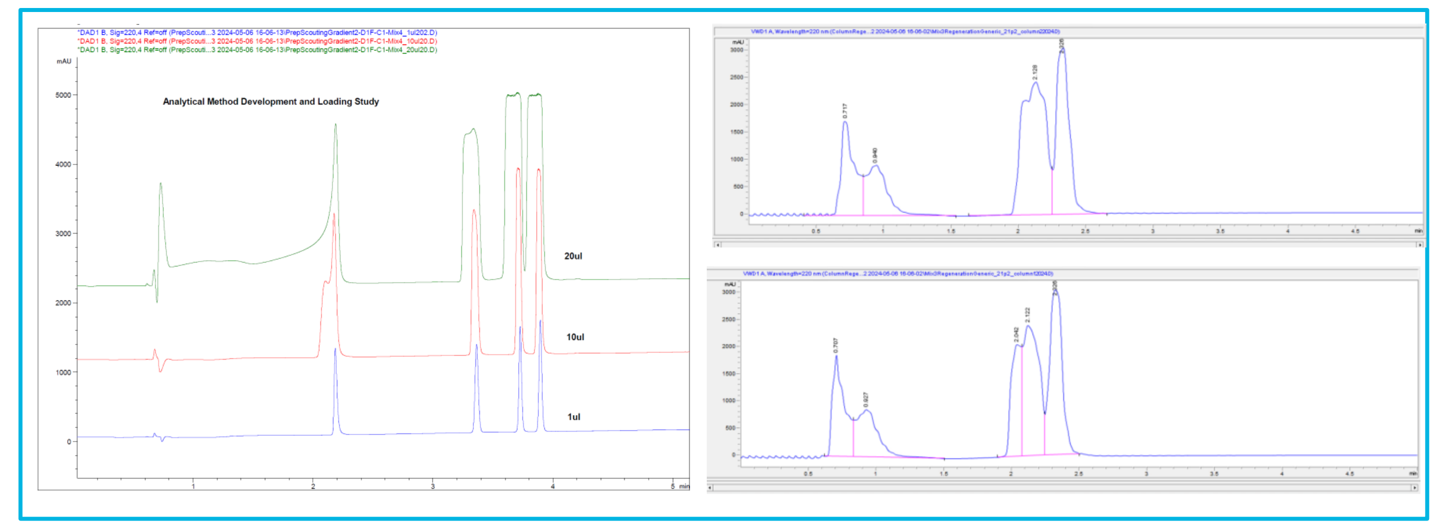

Fig. 1 Analytical Method Development, Loading Study for Scale Up, Preparative Injections

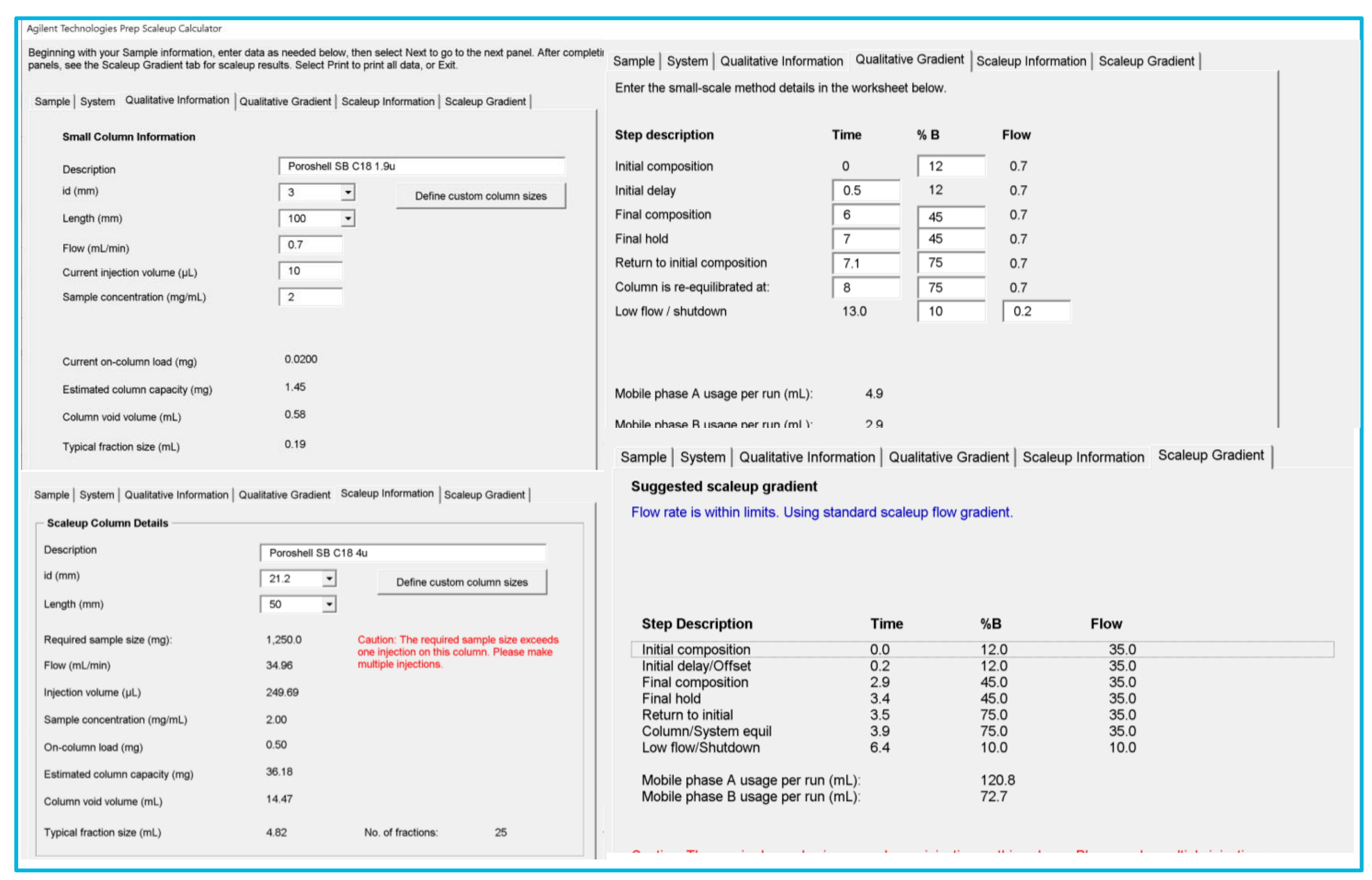

Fig. 2 Scale up Calculations

Results and Discussion

Purification Runs

To purify the 4 component mixture by preparative HPLC in a traditional setup, the method consists of the injection step, elution step, column cleanup step and column equilibration step. The pilot method optimized the separation on an analytical column with a 10ul injection volume. The elution gradient on the analytical column was 11 minutes, including the column cleanup and re-equilibration steps. The scaleup to the prep system and column was scaled to 10 minutes at a flowrate of 35ml/min with a 250ul injection. The 35 ml/min flow rate is much higher than the typical ideal flowrate for a 21.2mm id prep column, due to the low backpressure of the poroshell solid core technology. The addition of the second prep pump, 2/14 valve and identical preparative column allows the configuration to operate in an offline column regeneration mode in parallel to online column elution mode. By performing both processes in parallel the throughput was doubled and the cycle time was reduced to 5 minutes per run. It is speculated that by shortening the elution time further and ending the run after the peak of interest is eluted at 1.5 minutes, the throughput could be potentially tripled or more. The potential to perform the cleanup and equilibration steps at higher flowrates due to the low back pressure of the Poroshell column could further reduce the offline column steps.

Results and Discussion

Method Programming

Chemstation Software was used to program the elution method as well as the offline column regeneration and valve control. The standard elution method was programmed for pump 1 with a stop time to control the entire method. The pump 1 program included the linear scale up of the flow rate from 0.7ml/min on the 3x100mm analytical column to 35ml/min on the 21.2x50mm preparative column. These flowrates would not normally be achieved on a fully porous packing material, thus the use of the poroshell columns has further increased the throughput. Following the linear scale up equations in the Agilent Preparative Scale up Calculator will yield a predictable elution order and retention time for the preparative injections. The cleanup and equilibration steps were moved to the method program for Pump 2, the regeneration pump. As this part of the method was only for clean up and not for separation, unmodified solvents were used. The regeneration pump was run at 40ml/min as this was well under the maximum column and system pressure and yielded a faster regeneration method. Thorough cleanup of the column after each injection, extends the lifetime of the column and yields a more robust method.

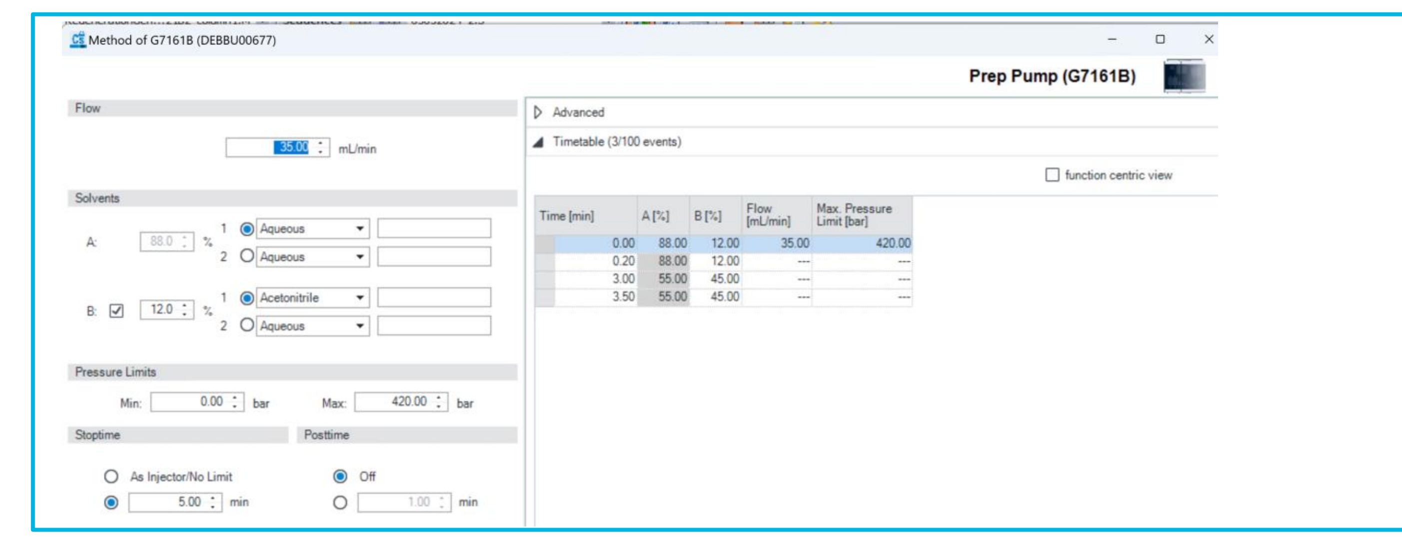

Fig. 3 Preparative HPLC Pump Method

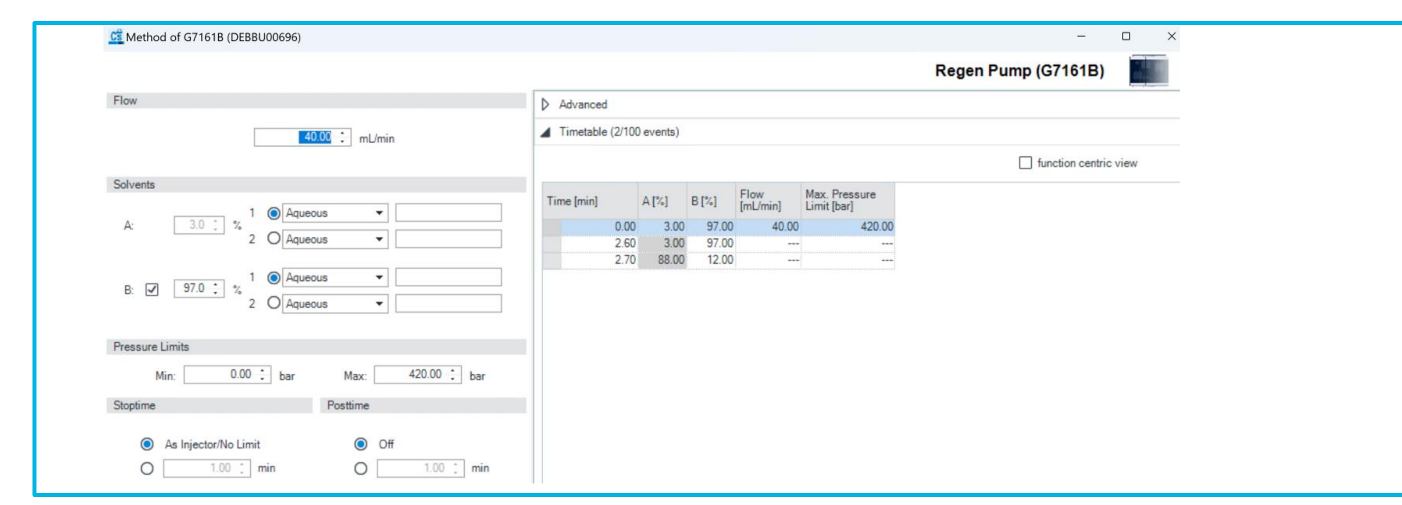

Fig. 4 Preparative HPLC Regeneration Pump Method

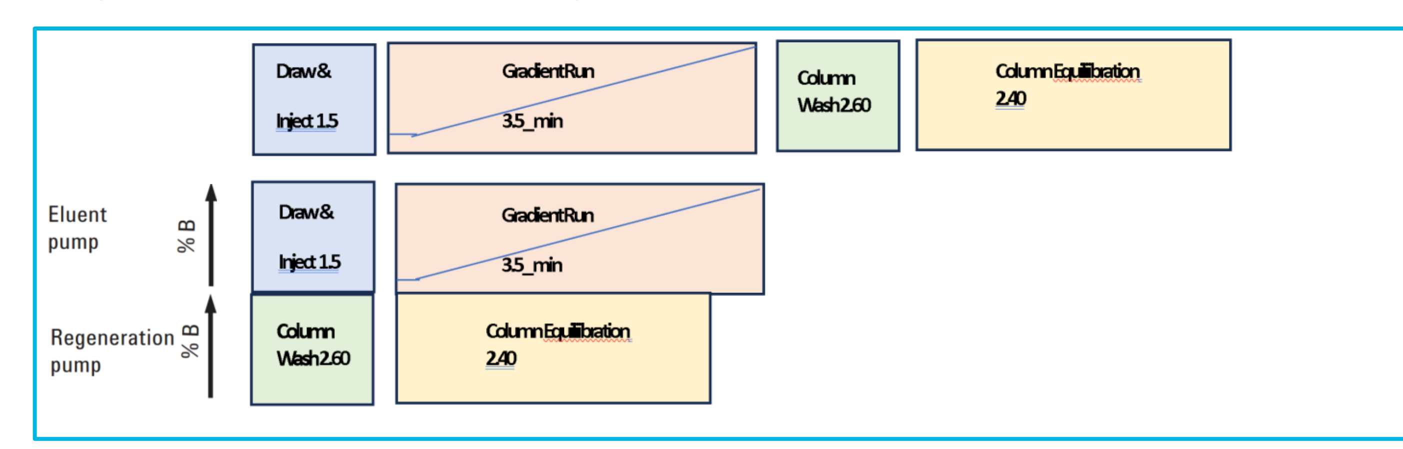

Fig. 5 Cycle Time for Sequential Method vs. Offline Parallel Column Regeneration Method

Conclusions

Faster run times and higher throughput is desirable when doing preparative HPLC. Using higher flow rates, shorter and lower pressure columns as well as optimized equipment can help to achieve these goals. Higher backpressure limits of the Agilent Inf II Prep pumps along with high pressure valves further reduce cycle times by allowing configuration of automated offline column regeneration. This means two columns are alternatively used via a 2-position/14-port valve. Cleaning and equilibration time can be excluded from the cycle time and 50% more runs were performed.

References

References:

Udo Huber, "High throughput HPLC — Alternating column regeneration with the Agilent 1100 Series valve solutions", Agilent Technologies Application Note, 2002, publication number 5988-7831

A.G.Huesgen and Edgar Naegele, "Automated alternating column regeneration on the Agilent 1290 Infinity LC", Agilent Technologies Application Note, 2009, publication number 5990-5069EN