Automated Small Scale Protein Purification for Bio-analytical Characterization Workflows

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1 Overview

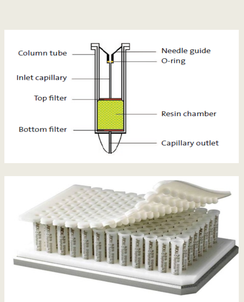
Performance reproducibility and reliable linear scalability from small to process-scale is critical to the viability of pharmaceutical and biotechnology company product pipelines. Optimization of purification conditions and characterization of the reproducibility of separation media is an area of increasing importance to successful development of protein therapeutics and vaccines.

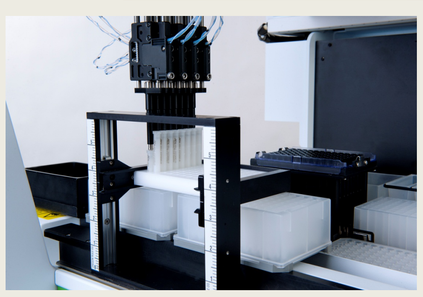
We present a study using automated small scale purification to reproducibly qualify pre-packed column purification resins utilizing standard chromatography qualification HEPT (height equivalent of a theoretical plate) and DBC (dynamic binding capacity) protocols.

2 Instrumentation

JANUS BioTx Pro:

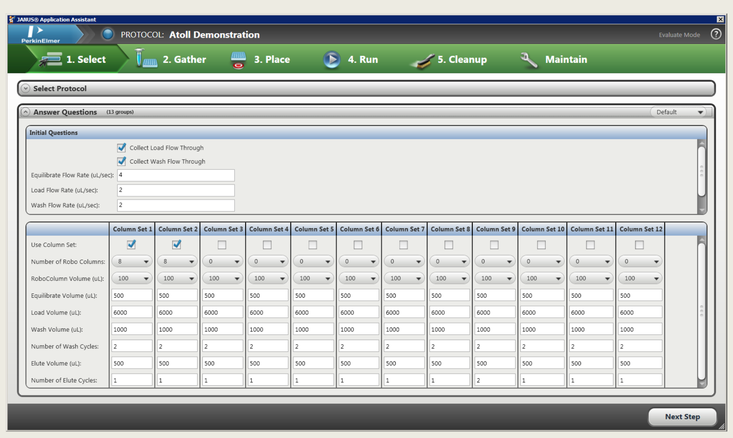
- 12 deck positions
- 8-tip dispense arm with Varispan™
- Pipetting with both fixed tips and disposable tips
- plate::shuttle for automated fraction collection



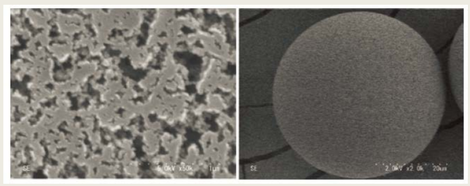


VersaTip fixed tip with a modifies sample loop and plate::shuttle for automating MediaScout® RoboColumns with bed sizes of 50-600µL.

An intuitive user interface enables rapid iterations of variables for optimization of purification techniques.



JSR Micro Amsphere™ Protein A is a novel resin designed for the large-scale downstream processing of bio-molecules. The resin was developed with JSR™s proprietary technology using a design of hydrophilic and porous resins with large surface area.



3 Proof-of-Concept Applications

MediaScout® RoboColumns column sizes: 200µL columns and 600µL columns. For each column size, 4 independent sets of 8 columns were tested.

HETP Protocol: Goal was to evaluate the column clearance efficiency by height equivalent of a theoretical plate and peak asymmetry. Fraction spread was identified based on the UV peak from the load buffer.

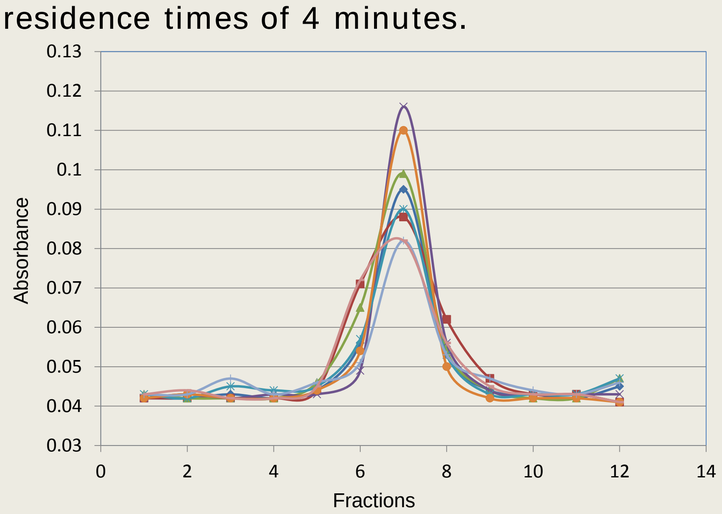
The columns were equilibrated with running buffer, followed by a load of a small volume of acetone containing buffer. Fractions (100µl) were eluted using running buffer, and collected in a 96-well plate for UV measurement.

DBC Protocol: Goal was to establish the breakthrough capacity for a particular pAb for each of the respective MediaScout® RoboColumns column sizes.

Running buffer with pAb was passed over the column. After column saturation, the pAb appeared in the collection fractions, providing the break-through capacity of the column for the particular pAb.

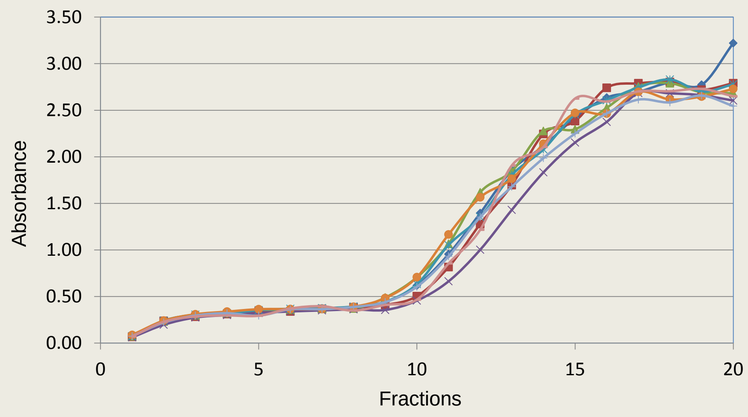
5 Results

HETP Protocol:
15µL injection of 2% acetone buffer, for the 600µL columns with collections of 100µL size fractions. Volumetric flow rates of 0.84 µL/sec were used to achieve residence times of 4 minutes.

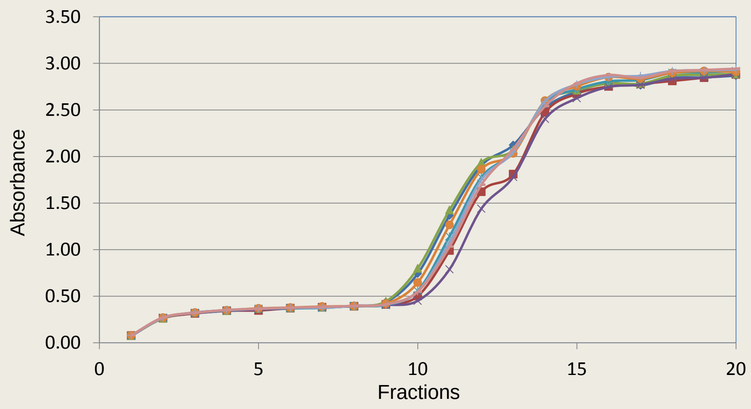


The HETP Protocol demonstrates effective clearance of the MediaScout® RoboColumns column. The acetone buffer spike is clearly visible and was captured in fraction 7.

DBC Protocol:
Equilibrated 200µL columns were loaded with 4mL of pAb for complete saturation at flow rates of 0.84 µL/sec (4 minutes residence time). Fractions of 200µL were collected.



Equilibrated 600µL columns were loaded with 12mL of pAb for complete saturation at volumetric flow rate of 2.5 µL/sec (4 minutes residence time). Fractions of 600µL were collected.



The curves show the break-through point around fraction 10 (10 x 200µL = 10CV and 10 x 600µL = 10CV, respectively). Column saturation was observed around fraction 18.

6 Summary

The JANUS BioTx Pro workstation was successfully demonstrated on evaluation of the column performance of Amsphere™ (JSR Life Sciences) Protein A RoboColumns. This fully flexible and scalable system could thus be used for automated processing of micro-scale chromatography columns.

The JANUS BioTx Pro could offer significant time savings in the process development of bio-pharmaceuticals.