APPLICATION NOTE

Viral Clearance under Mild pH using Protein A Resins

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Introduction

Host Protein A affinity resins are essential for purifying monoclonal antibodies (mAbs) and similar therapeutic molecules. During the purification process, the target molecule binds to the affinity column, and a lower pH is typically used for elution. However, using typical elution levels (pH 3–pH 3.5) can result in sensitivity issues, often manifested as increased aggregation or other unwanted modifications — significantly reducing yield.

Study Overview

In this study, the performance of two Purolite™ chromatography resins, Praesto Jetted A50 and Praesto Jetted A50 HipH, was compared to that of a commercially available agarose-based protein A resin (Competitor A).

This study evaluated the viral clearance capabilities of Praesto Jetted A50 under standard protein A elution conditions, as well as Praesto Jetted A50 HipH at both standard and milder elution levels. The viruses and experimental conditions utilized in this study were chosen based on industry guidelines (ICH Q5A), with the goal of determining log reduction values (LRV) for viral clearance for two model viruses: one enveloped (MLV) and one non-enveloped (MMV).

A total of sixteen runs were performed, two per virus across each elution condition studied. Duplicate runs for each virus and condition were conducted to verify the consistency of the reduction factors. Consistency was shown by the log reduction factors of each duplicate run being within one log of each other.

TABLE 1 Summary of Viral Clearance Runs

Summary of runs performed and elution conditions.

Resin	Elution	MLV	*MMV*
Praesto Jetted A50	pH 3.5	2	2
Praesto Jetted A50 HipH	pH 3.5	2	2
Praesto Jetted A50 HipH	pH 4.5	2	2
Competitor A	pH 3.5	2	2
	TOTAL RUNS/VIRUS	8	8

TABLE 2 Chromatography Column Parameters

Parameter	Small-Scale Model
Column Diameter (cm)	1.0
Column Bed Height (cm)	20
Column Volume	15.7 mL
Linear Flow Rate (cm/h)	300 for process 200 for loading
Volumetric Flow Rate	3.93 mL/min for process 2.62 mL/min for loading
Load Concentration (g/L)	2.8
Load Ratio	45 g of product per L of resin

TABLE 3 Small-Scale Method for Viral Clearance Study

Step	Solution	Target Volume (CV)	Flow Rate (cm/h)	Estimated Time (mins)
Rinse	Purified Water	2	160	15
Pre-Use Sanitization	0.5 M Sodium Hydroxide	3	240	15
Rinse	Purified Water	2	300	8
Neutralization	20 mM Sodium phosphate, 150 mM sodium chloride, pH 7.4	2	300	8
Equilibration	20 mM Sodium phosphate, 150 mM sodium chloride, pH 7.4	4	300	15
Load	Pro A Load Target load ratio: 45 g/L	Variable	200	Variable
Post Load Wash 1	20 mM Sodium phosphate, 500 mM sodium chloride, pH 7.4	1.5	200	9
		3.5	300	14
Post Load Wash 2	50 mM Tris, 1 M sodium chloride pH 8.0	5	300	20
Post Load Wash 3	50 mM Sodium phosphate, pH 7.0	3	300	12
Elution	50 mM Sodium acetate, pH 3.5 Or 50 mM Sodium acetate pH 4.5	6	300	24
Strip	100 mM Acetic Acid	2	300	8
Rinse	Purified Water	2	300	12

TABLE 4 Total Virus Loaded for Small-Scale Model Runs

Resin	MMV (by TCID50)	MLV (by QPCR)
Praesto Jetted A50	8.34 ± 0.36 log10	11.35 ± 0.04 log10
Praesto Jetted A50 HipH (Elution pH 3.5)	8.43 ± 0.33 log10	11.40 ± 0.04 log10
Praesto Jetted A50 HipH (Elution pH 3.5)	8.25 ± 0.38 log10	11.39 ± 0.04 log10
Competitor A	8.52 ± 0.28 log10	11.23 ± 0.04 log10

Viral Clearance Results

Overlays of the UV A280nm profile of the chromatographs for the MLV-spiked runs are shown in Figures 1–4 and MMV-spiked runs are shown in Figures 5–8. Each figure includes both runs performed at that elution condition (first in blue, second in red).

Figure 1

MLV-Spiked Run for Praesto Jetted A50 at pH 3.5

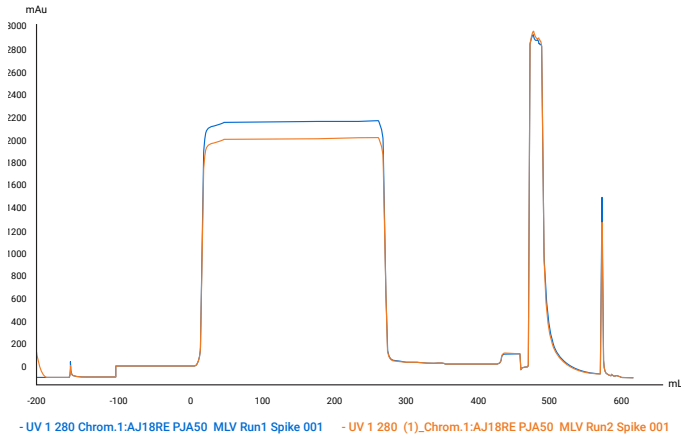


Figure 2

MLV-Spiked Run for Praesto Jetted A50 HipH at pH 3.5

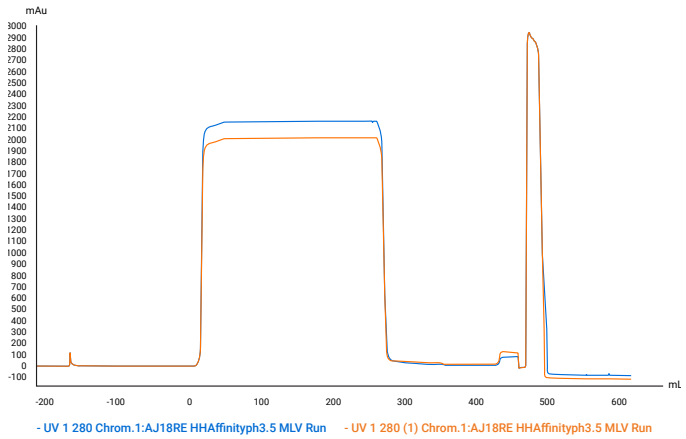


Figure 3

MLV-Spiked Run for Praesto Jetted A50 HipH at pH 4.5

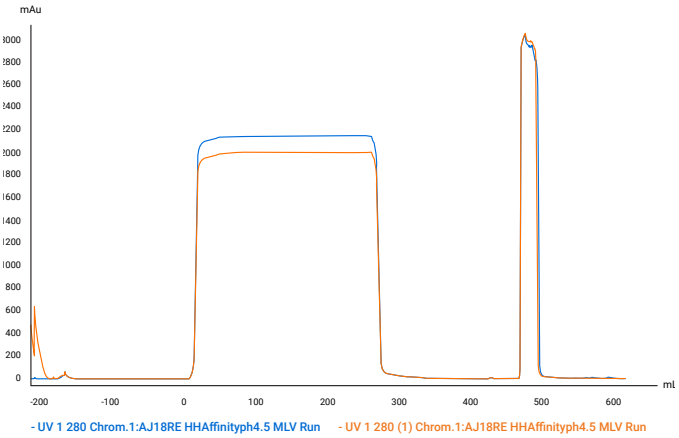


Figure 4

MLV-Spiked Run for Competitor A at pH 3.5

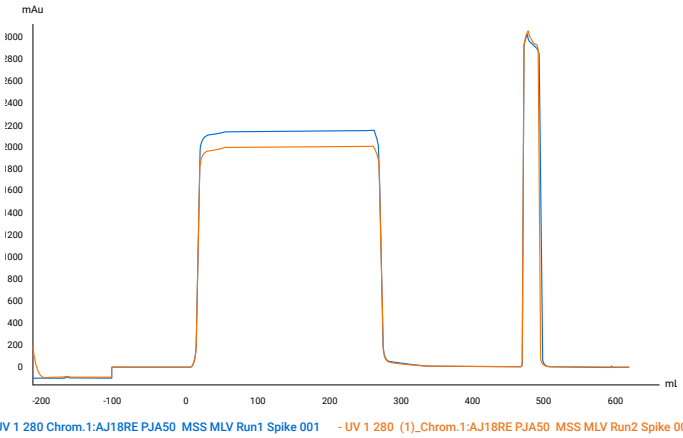


Figure 5

MMV-Spiked Run for Praesto Jetted A50 at pH 3.5

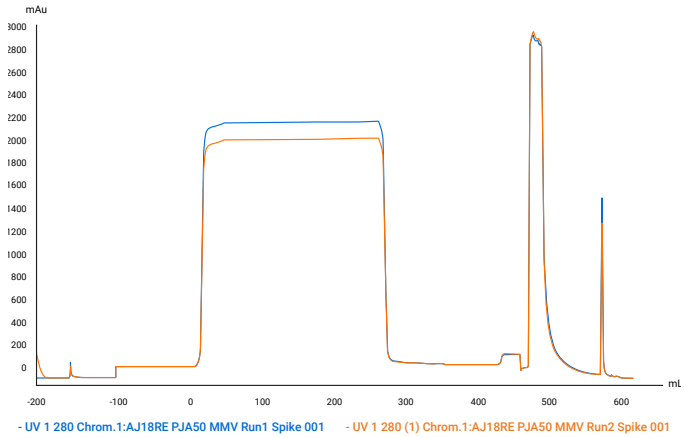


Figure 6

MMV-Spiked Run for Praesto Jetted A50 HipH at pH 3.5

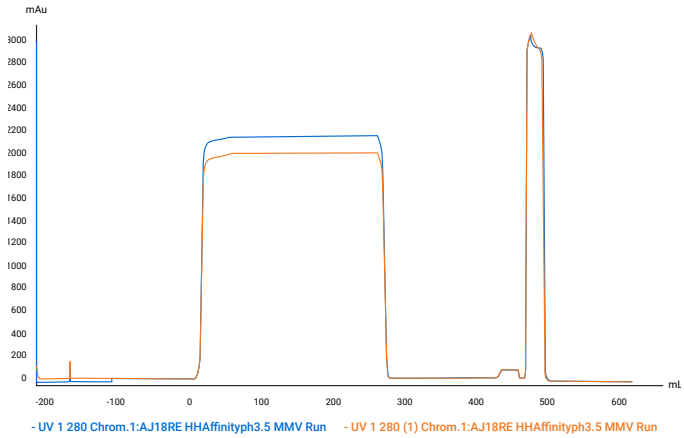


Figure 7

MMV-Spiked Run for Praesto Jetted A50 HipH at pH 4.5

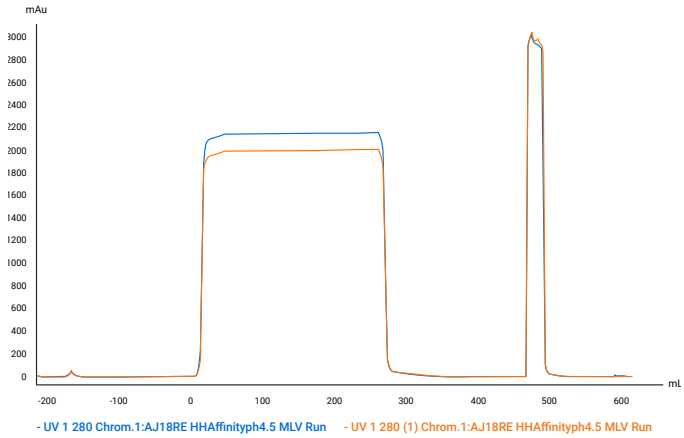
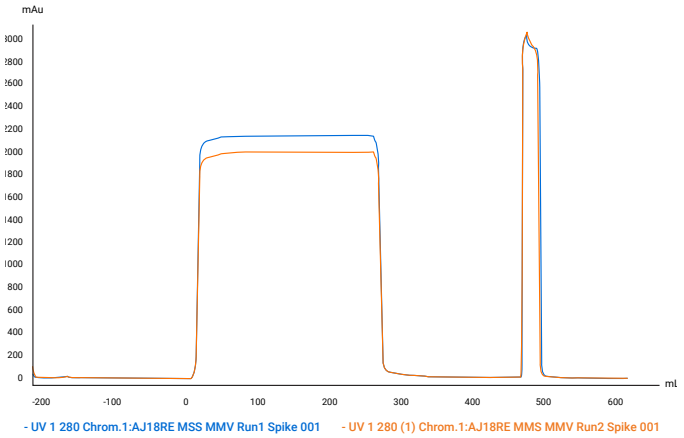


Figure 8

MMV-Spiked Run for Competitor A at 3.5



Summary

Viral clearance results indicate significant clearance across each protein A chromatography resin studied — with all resins showing a log reduction factor greater or equal to 2 log10. Duplicate runs proved consistent as all duplicate runs remained within 1 log of each other. In this study, Praesto Jetted A50 HipH exhibited better removal of both viruses compared to Praesto Jetted A50 and the competitor resin. For MLV, the virus log reduction of Praesto Jetted A50 HipH with high elution pH 4.5 showed better removal (log reduction of 4.85 ± 0.06 log10) compared to any run using the typical elution of pH 3.5. For MMV, the virus log reduction of Praesto Jetted A50 HipH with high elution pH 4.5 showed better removal (log reduction of 3.85 ± 0.52 log10) compared to any run using the typical elution of pH 3.5.

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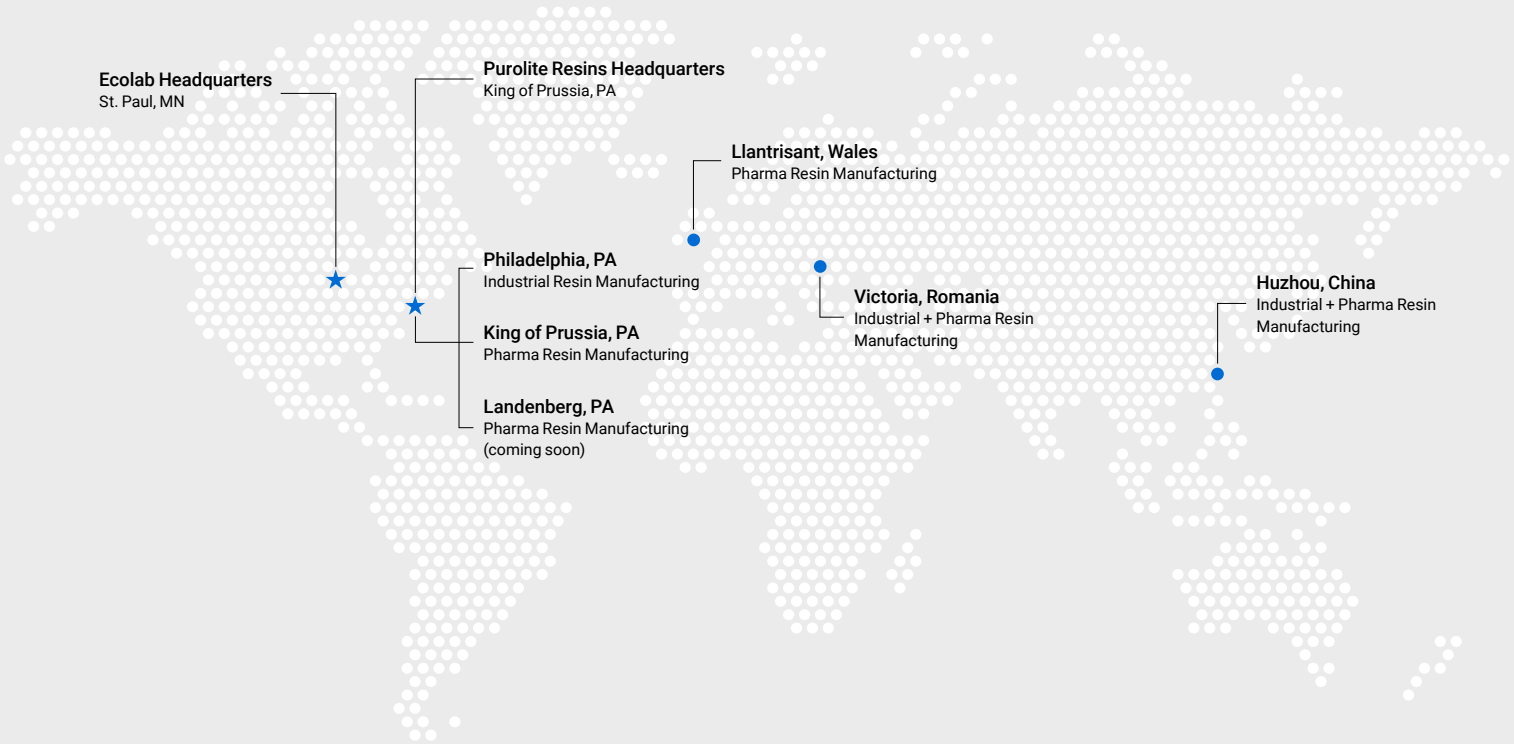

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