Millipore®
Preparation, Separation,
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Technical Brief

# Citric Acid or Acetic Acid?

## Understanding the impact of elution buffer on mAb purification processes

### Introduction

Successful affinity chromatography relies on a specific interaction between the target molecule and the chromatography resin, as well as the ability to separate the product from the resin using an elution buffer. In Protein A affinity chromatography, process developers need to choose from a range of available elution buffers and conditions to implement at production scale. Elution buffer choice can significantly impact the characteristics of the elution pool and the subsequent downstream steps.

This study explores the impact of a range of elution buffer conditions, including molarity (0.01 M–0.1 M), pH (3.0–3.7) and buffer type (citric acid and acetic acid), on two different commercial Protein A resins (Eshmuno® A and ProSep® Ultra Plus affinity resins). Measured outcomes included antibody yield, chromatographic profile, pool pH and volume of the elution pool. The effects of elution buffer concentration and pH on these parameters were evaluated. Many factors need to be considered when selecting an elution buffer, and this work demonstrates the impact elution buffer choice can have on the efficiency and process robustness of mAb purification.




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## Experimental Methods

Varying concentrations of two elution buffers (citric acid and acetic acid) were used in a standard protein A chromatography methodology. The elution buffer concentration were as follows:

| Concentration (mM) | pH |
|---|---|
| **Citric acid buffers** | |
| 100 | 3.0 |
| 100 | 3.5 |
| 100 | 3.7 |
| 50 | 3.0 |
| 50 | 3.5 |
| 50 | 3.7 |
| 20 | 3.0 |
| 20 | 3.5 |
| 20 | 3.7 |
| 10 | 3.0 |
| 10 | 3.5 |
| 10 | 3.7 |

| Concentration (mM) | pH |
|---|---|
| **Acetic acid buffers** | |
| 10 | 3.5 |
| 100 | 3.5 |
| 100 | 3.7 |
| 50 | 3.0 |
| 50 | 3.5 |
| 50 | 3.7 |
| 20 | 3.0 |
| 20 | 3.5 |
| 20 | 3.7 |

### Process Steps for Protein A Chromatography

| Step | Buffer | CV | RT (min.) |
|---|---|---|---|
| Strip | Same as Elution | 3 | 3 |
| Equilibration | 50 mM Tris 25 mM NaCl 5 mM EDTA pH 7 | 7 | 3 |
| Load | 4 mg/mL Polyclonal IgG in EQ buffer | Load to 32 mg/mL | 3 |
| Wash | 50 mM Tris 25 mM NaCl 5 mM EDTA pH 7 | 4 | 3 |
| Intermediate Wash | 0.1 M Citric Acid pH 5.5 | 4 | 3 |
| Wash | 50 mM Tris 25 mM NaCl 5 mM EDTA pH 7 | 3 | 3 |
| Elution | Varies | 8 | 6 |
| Wash | 50 mM Tris 25 mM NaCl 5 mM EDTA pH 7 | 4 | 3 |
| Strip | 6M Guanidine HCl | 3 | 3 |
| Equilibration | 50 mM Tris 25 mM NaCl 5 mM EDTA pH 7 | 5 | 3 |

Experiments used either ProSep® Ultra Plus or Eshmuno® A affinity resins packed into Omnifit® columns with 1 cm i.d. X 5 cm bed height. Both resins were tested under all of the above experimental conditions, with one resin duplicated for each condition. The feed was human polyclonal IgG. Elution peaks were collected between 100 mAU and 200 mAU at UV 280 nm, and analyzed for yield, conductivity, pH and volume.

## Results and Discussion

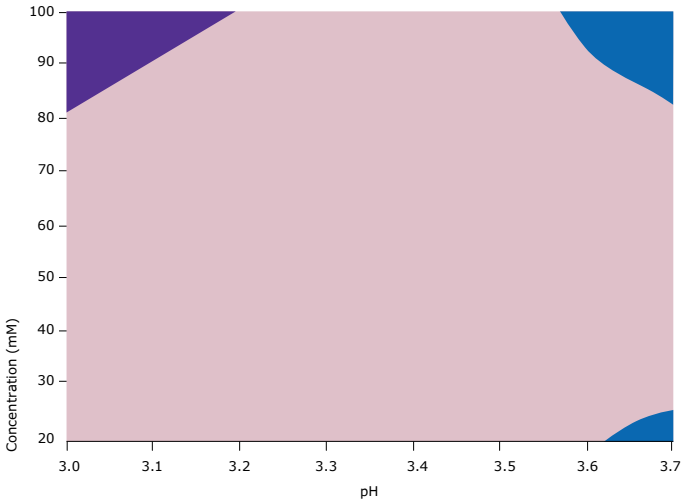
Citric acid and acetic acid were used separately as elution buffers with two different Protein A resins: ProSep® Ultra Plus and Eshmuno® A affinity resins. The results were then compared side-by-side to determine the optimal buffer and pH for affinity chromatography. Product yields (not shown) were consistently within the acceptable range for all conditions explored.

## Elution Pool Volume

The first criterion for comparison was elution pool volume. As seen in Figure 1, citric acid buffers resulted in consistent elution pool volumes (less than 1 CV variation) across a range of pH and salt concentration values for both ProSep® Ultra Plus and Eshmuno® A resins. In contrast, acetic acid buffers resulted in greater variation (>1 CV) of the elution pool volumes for both affinity resins. This is likely due to the lower buffering capability of acetic acid.

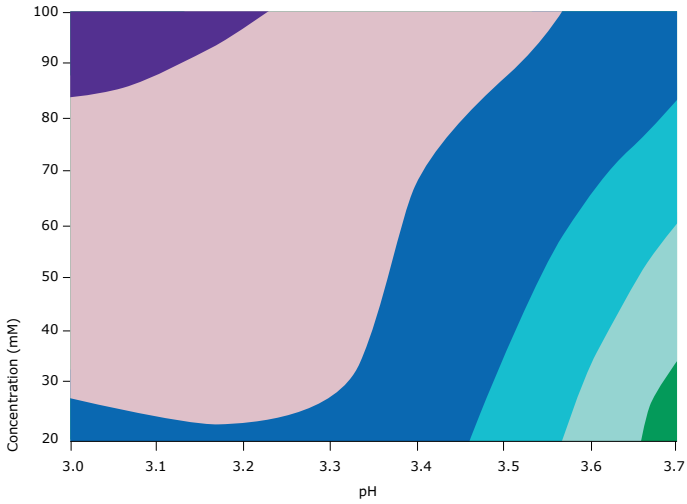
**A. ProSep® Ultra Plus Resin**

Contour plot of elution volume (CV) vs. concentration (mM), pH



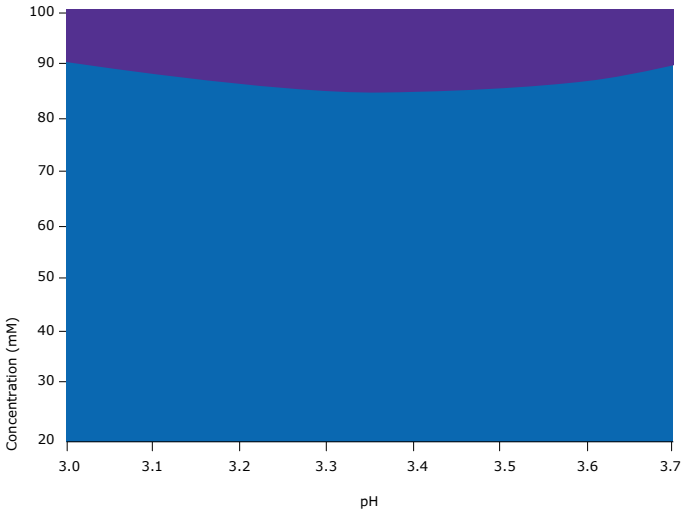

**A. Eshmuno® A Resin**

Contour plot of elution volume (CV) vs. concentration (mM), pH



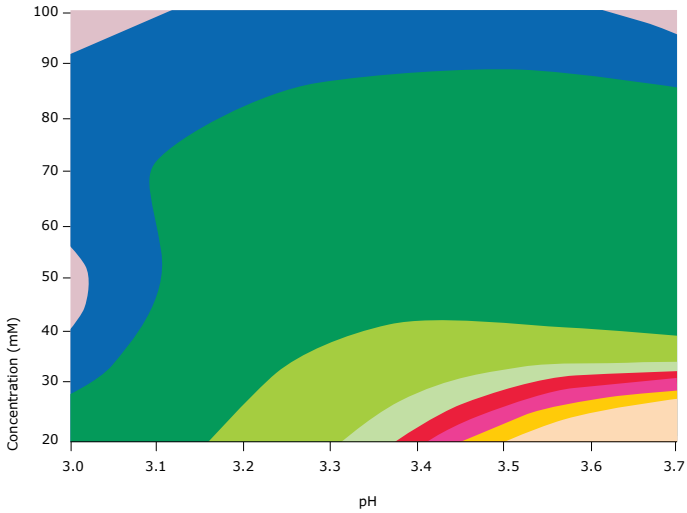

**B. ProSep® Ultra Plus Resin**

Contour plot of elution volume (CV) vs. concentration (mM), pH




**B. Eshmuno® A Resin**

Contour plot of elution volume (CV) vs. concentration (mM), pH




**Elution volume (CV)**

| | | | |
|---|---|---|---|
| < 1.50 | 2.00 – 2.25 | 2.75 – 3.00 | 3.50 – 3.75 |
| 1.50 – 1.75 | 2.25 – 2.50 | 3.00 – 3.25 | 3.75 – 4.00 |
| 1.75– 2.00 | 2.50 – 2.75 | 3.25 – 3.50 | > 4.00 |

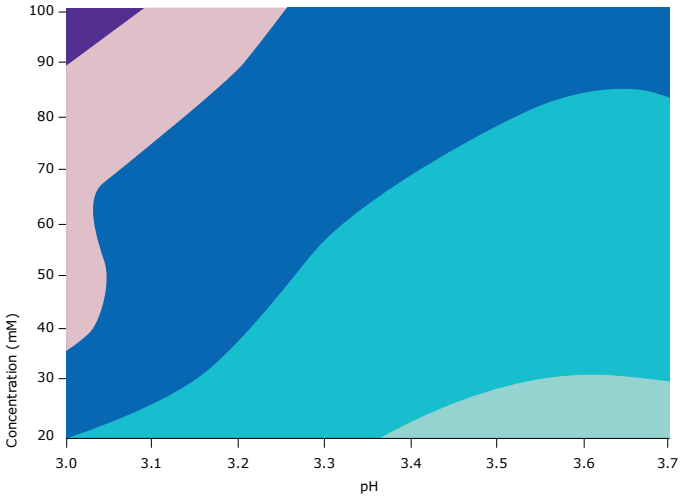
**Figure 1.** Elution pool volume using citric acid (A) and acetic acid (B) as the elution buffers on ProSep® Ultra Plus (left) and Eshmuno® A (right) affinity resins.

## Elution Pool pH

A second set of data identified the optimal pH range for each elution buffer. For citric acid (Figure 2A), the pH of the elution pool decreased as elution buffer molarity increased. ProSep® Ultra Plus affinity resin appeared to maintain a lower elution pH than Eshmuno® A affinity resin over the range of conditions tested. But, the general trend for the two resins was consistent. For acetic acid, the elution pool pH was relatively high at lower acetic acid molarity for both resins. The actual elution pool pH for Eshmuno® A affinity resin does not follow the same general trend as that of citric acid. Acetic acid's volatility and low buffering capability are likely the cause of the observed inconsistency (Figure 2B).

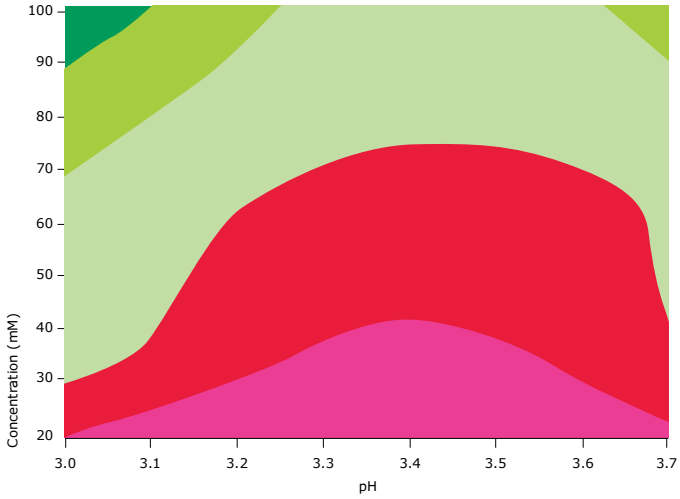
**A. ProSep® Ultra Plus Resin**

Contour plot of elution pool pH vs. concentration (mM), pH



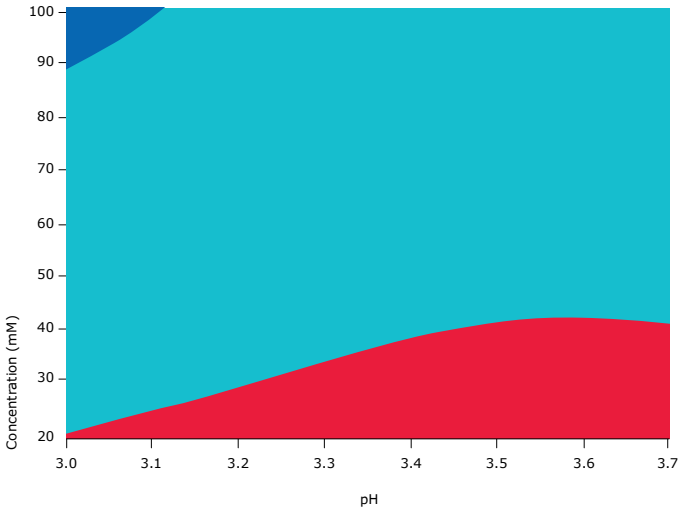

**A. Eshmuno® A Resin**

Contour plot of elution pool pH vs. concentration (mM), pH



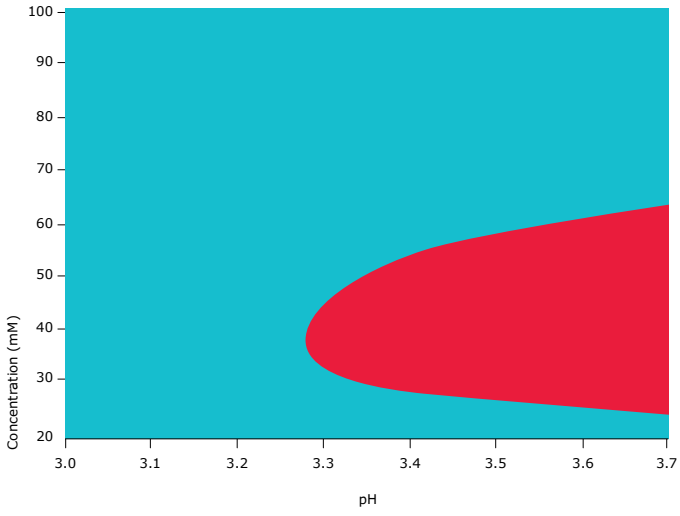

**B. ProSep® Ultra Plus Resin**

Contour plot of elution pool pH vs. concentration (mM), pH




**B. Eshmuno® A Resin**

Contour plot of elution pool pH vs. concentration (mM), pH




**Elution volume (CV)**

| | | | |
|---|---|---|---|
| < 1.50 | 2.00 – 2.25 | 2.75 – 3.00 | 3.50 – 3.75 |
| 1.50 – 1.75 | 2.25 – 2.50 | 3.00 – 3.25 | 3.75 – 4.00 |
| 1.75– 2.00 | 2.50 – 2.75 | 3.25 – 3.50 | > 4.00 |

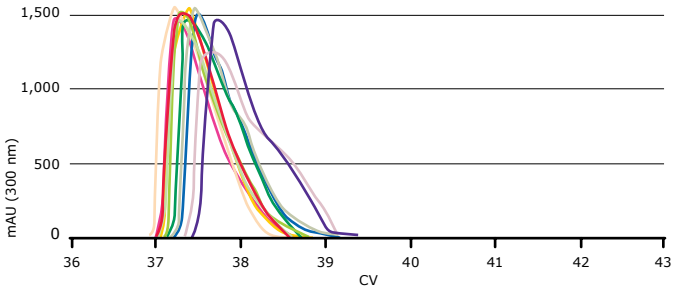
**Figure 2.** Elution pool pH using citric acid (A) and acetic acid (B) as the elution buffers on ProSep® Ultra Plus (left) and Eshmuno® A (right) affinity resins.

## Elution Peak Profile

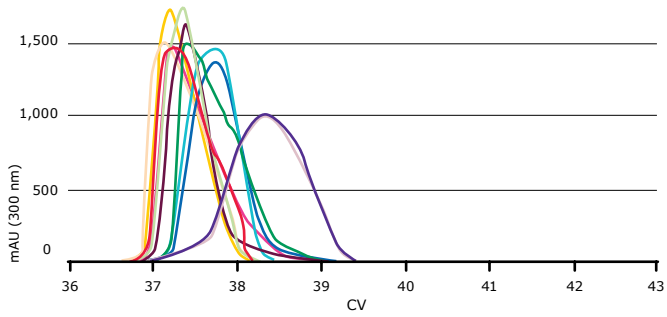
A third set of experiments compared the elution peak profiles for citric and acetic acids. Citric acid produced a relatively stable elution profile for both resins tested, at citric acid concentrations of 0.02 M and above (Figure 3A).

A broadening of the peak was observed at the lower concentration of 0.01 M on both resins (Figure 3A). By contrast, the elution profile on either resin was more scattered when acetic acid was employed as the elution buffer in comparison to that of citric acid (Figure 3B). Peak broadening and shoulders were observed at lower acetic acid concentrations or higher pH. Elution peaks were most consistent when acetic acid was at 0.1 M.

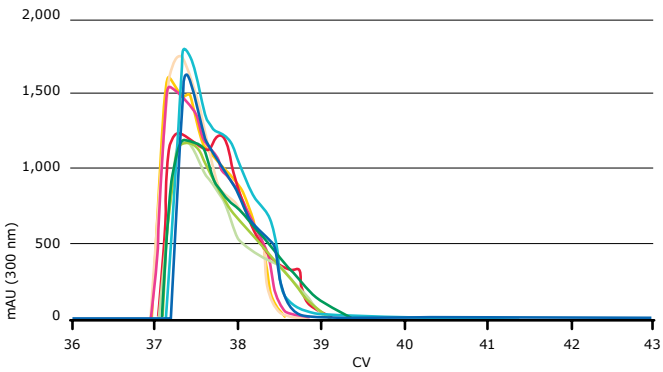
**A. ProSep® Ultra Plus Resin**
elution peak profiles with different citric acid elution conditions




**A. Eshmuno® A Resin**
elution peak profiles with different citric acid elution conditions




**B. ProSep® Ultra Plus Resin**
elution peak profiles with different citric acid elution conditions




**B. Eshmuno® A Resin**
elution peak profiles with different citric acid elution conditions

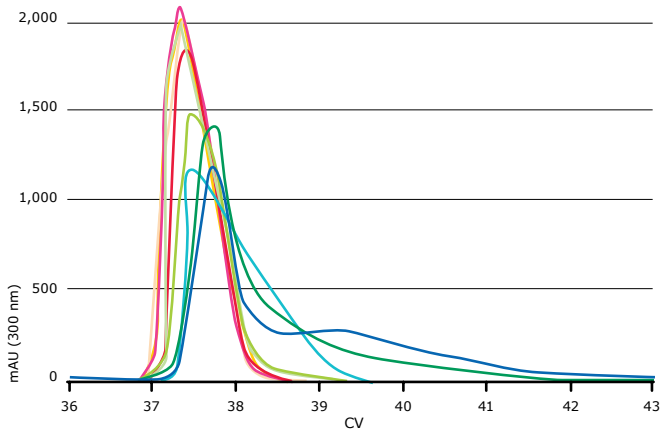




**Figure 3.** Elution peak profile using citric acid (A) and acetic acid (B) as the elution buffers on ProSep® Ultra Plus (left) and Eshmuno® A (right) affinity resins.

## Summary

Main effects plots were prepared using the results from all experiments in this study (Figure 4). The data indicated the elution volume was impacted by each variable studied. As elution conditions became stronger or the buffering capacity was increased, the elution volume decreased (Figure 4A). Further, the resulting elution pool pH was strongly impacted by the molarity of the elution buffer and type of elution buffer, and was not impacted by resin choice (Figure 4B). The increase in elution pool pH and elution buffer pH is not linear, and this might be attributed to other interactions, such as elution pool dilution and a buffering effect from the IgG itself.

**A. Main Effects Plot for Elution Volume (CV) Data Means**

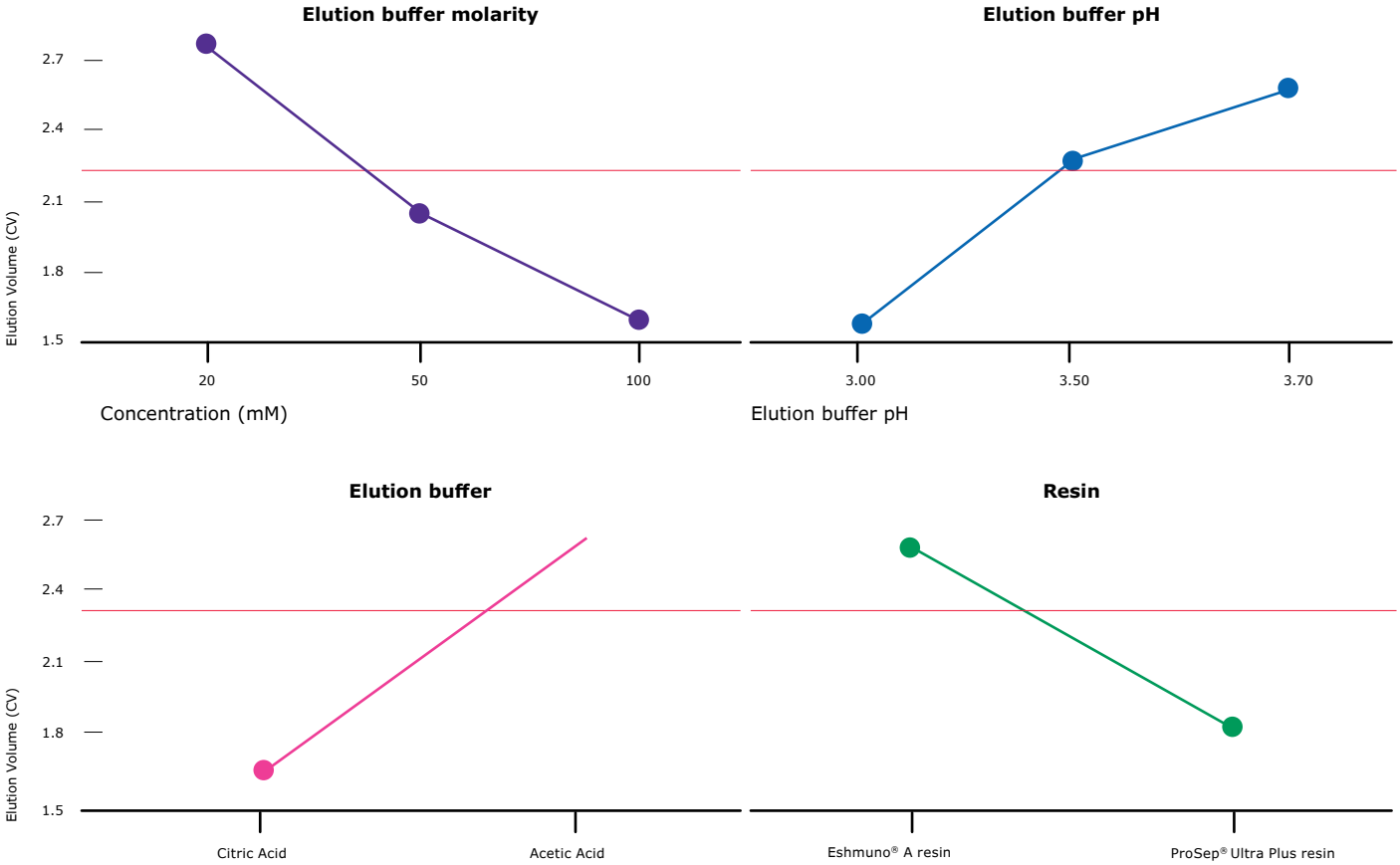



**Figure 4.** Main effects plots for this study, comparing elution volume CV (A) and elution pool pH (B).

**B. Main Effects Plot for Elution Pool pH Data Means**

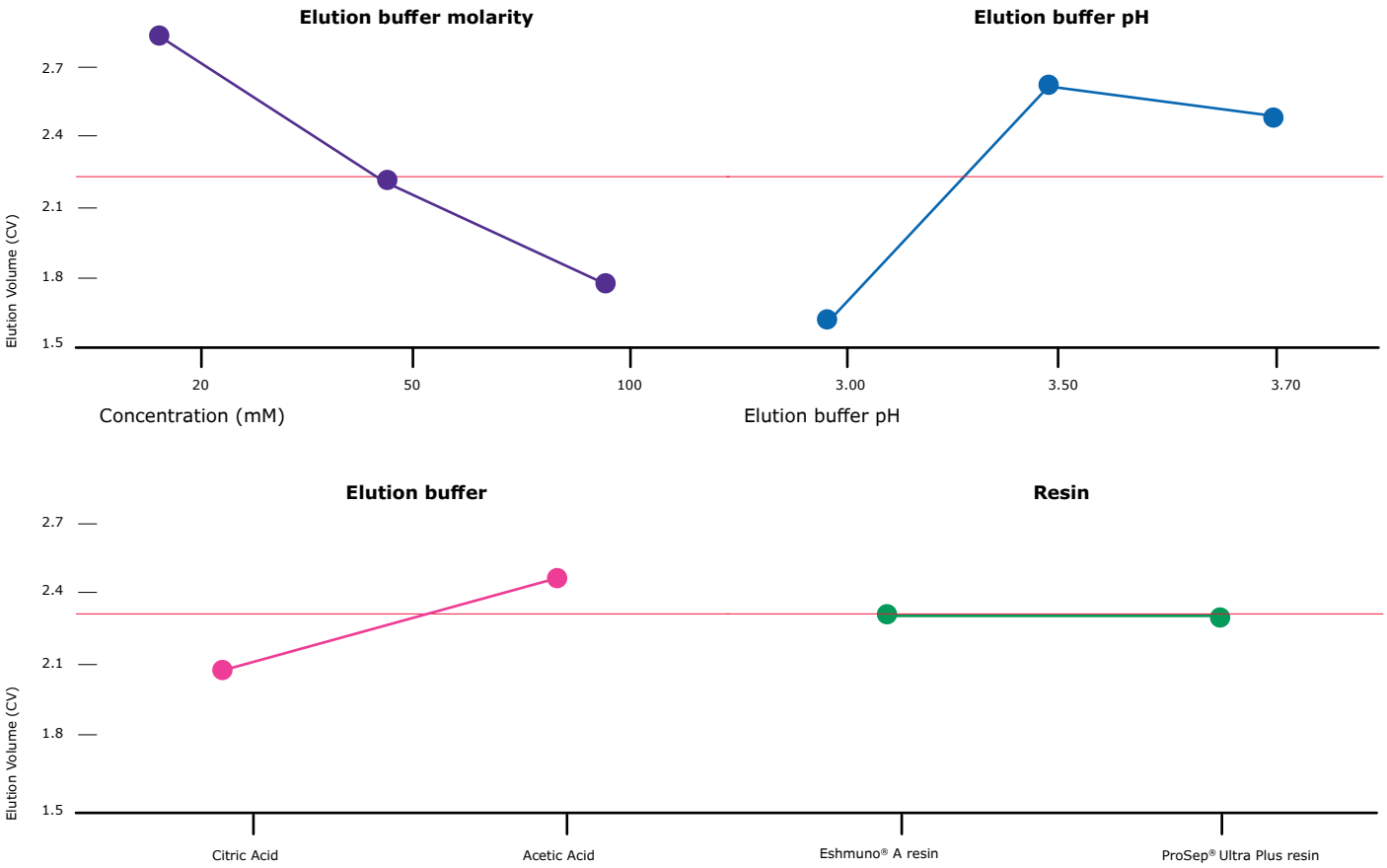



**Figure 4.** Main effects plots for this study, comparing elution volume CV (A) and elution pool pH (B).

## Conclusions and Recommendations

In this study, citric acid elution buffer provided a more consistent elution pool than acetic acid at comparable elution buffer pH for the resins tested. Citric acid's higher buffering capacity and lower volatility likely contribute to this phenomenon.

Although acetic acid of the same molarity typically generates lower conductivity elution pools, its higher volatility and lower buffering capacity should be considered when choosing a Protein A elution buffer for production. Many factors need to be considered when selecting an elution buffer, and this work demonstrates the impact elution buffer choice can have on the efficiency and process robustness of mAb purification.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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