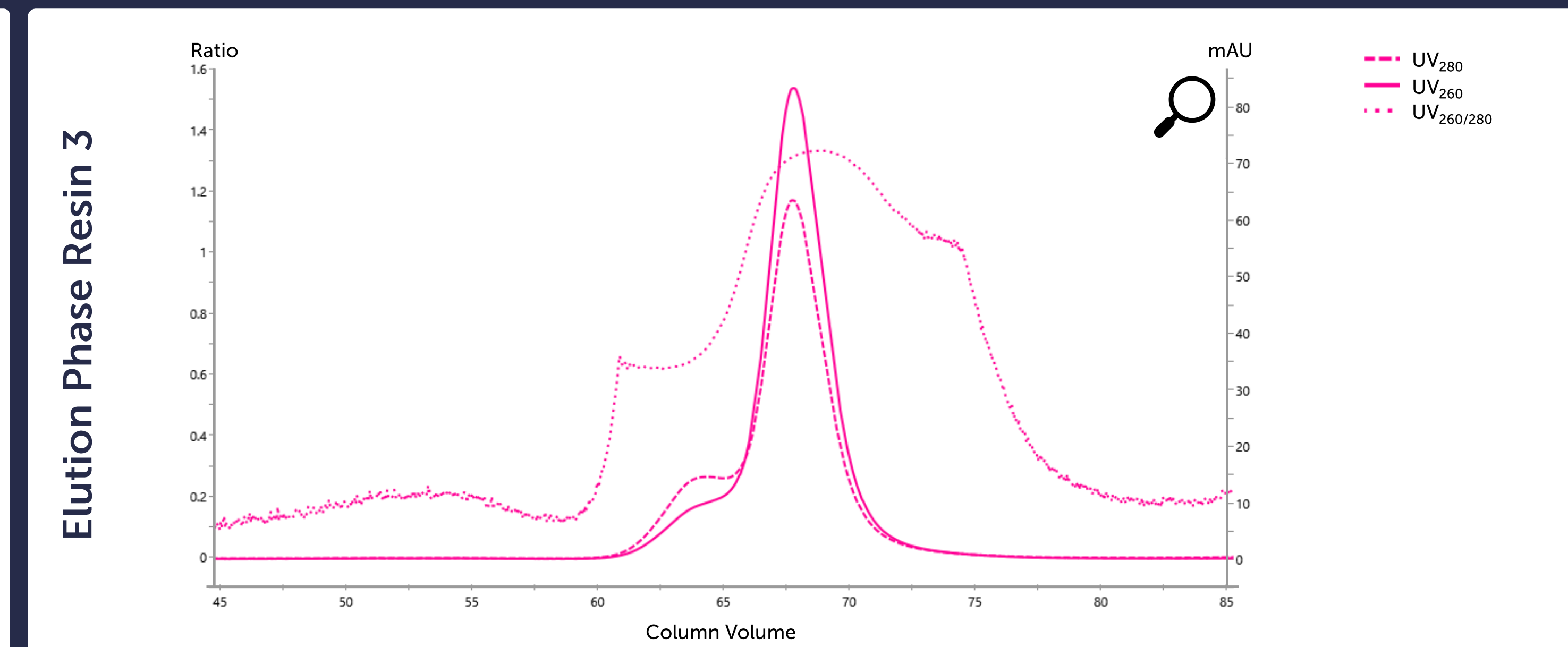
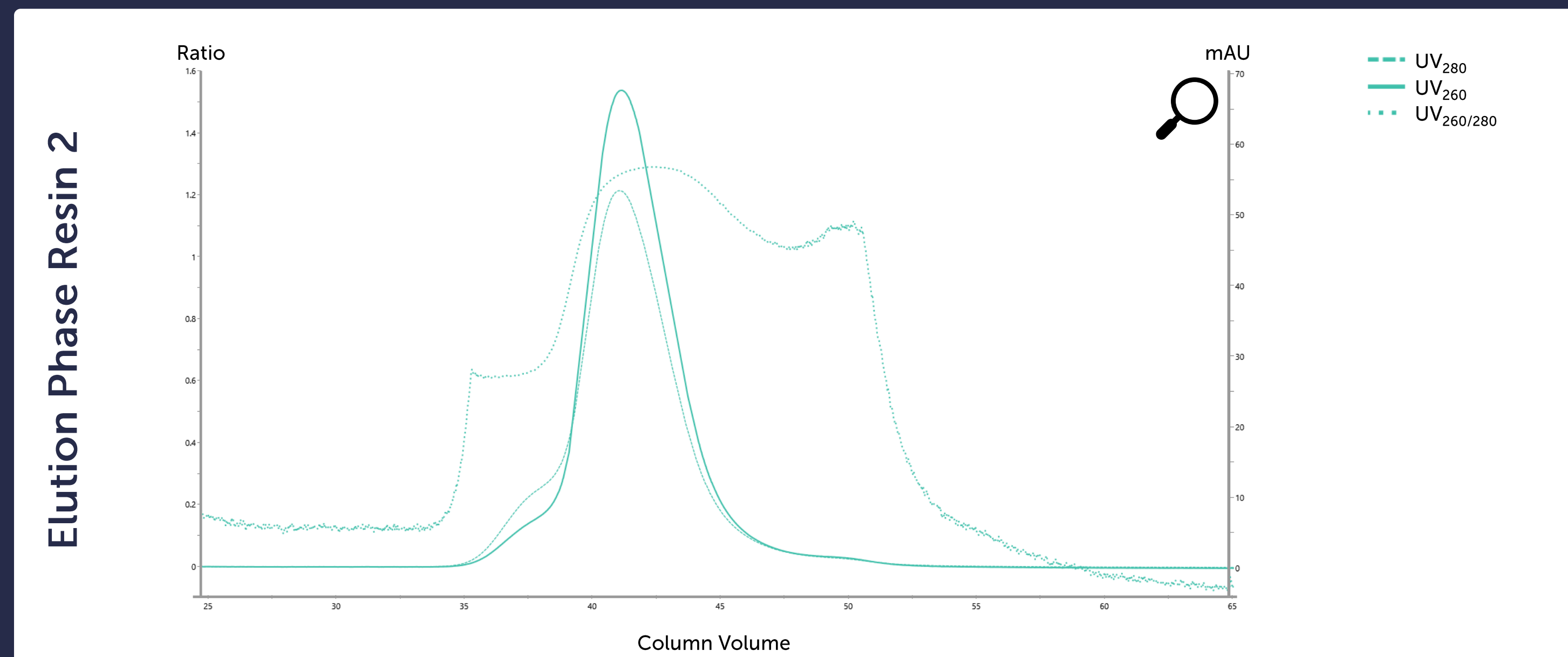
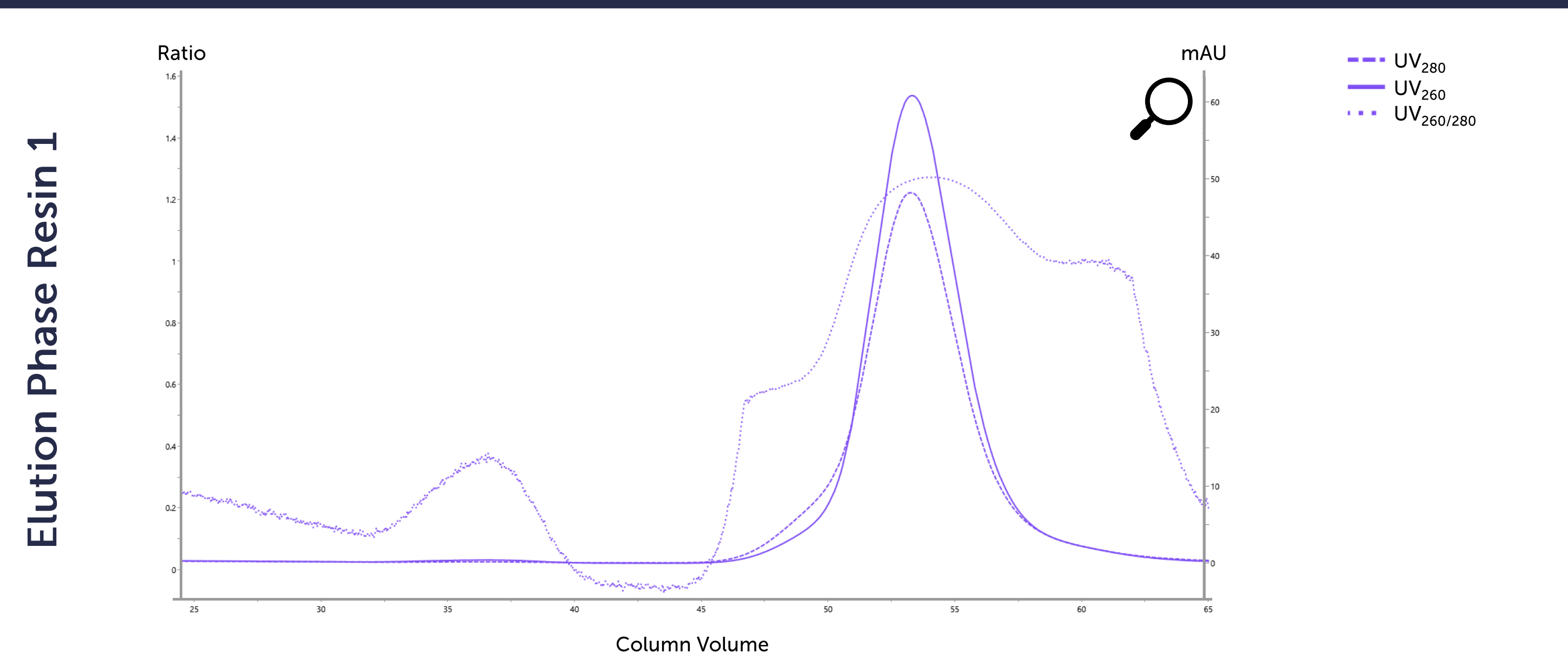


Unveiling the AAV8 Full Empty Separation Potential of Three Resins for Platform Innovation

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Introduction One of the central challenges in the production of AAV vectors for gene therapy is the efficient separation of full from empty capsids, which are unwanted by-products that can negatively impact efficacy and safety. As AAV gene therapy progresses towards clinical and commercial applications, efficient and scalable purification processes are becoming increasingly important.

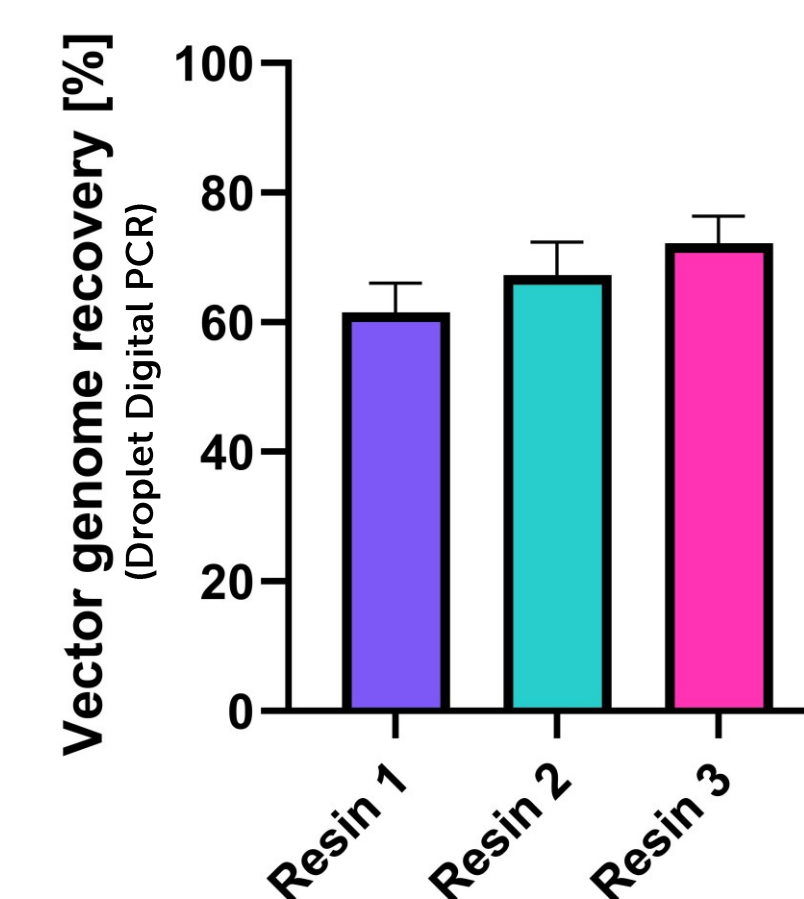
Materials The performance of three chromatographic resins was systematically evaluated with respect to their ability to separate full and empty AAV8 capsids under scalable anion exchange (AEX) chromatography conditions. Each resin was assessed based on vector genome recovery (by droplet digital PCR), host cell protein clearance (by Gyrolab immunoassay), total dsDNA impurities (by Qubit), full-to-empty separation efficiency (by mass photometry) and polydispersity index (by Unchained Labs Stunner). While buffer compositions and AEX parameters were optimized individually for each resin, eluate collection was standardized by applying consistent UV absorbance ratio-based fractionation criteria to enable a direct comparison.



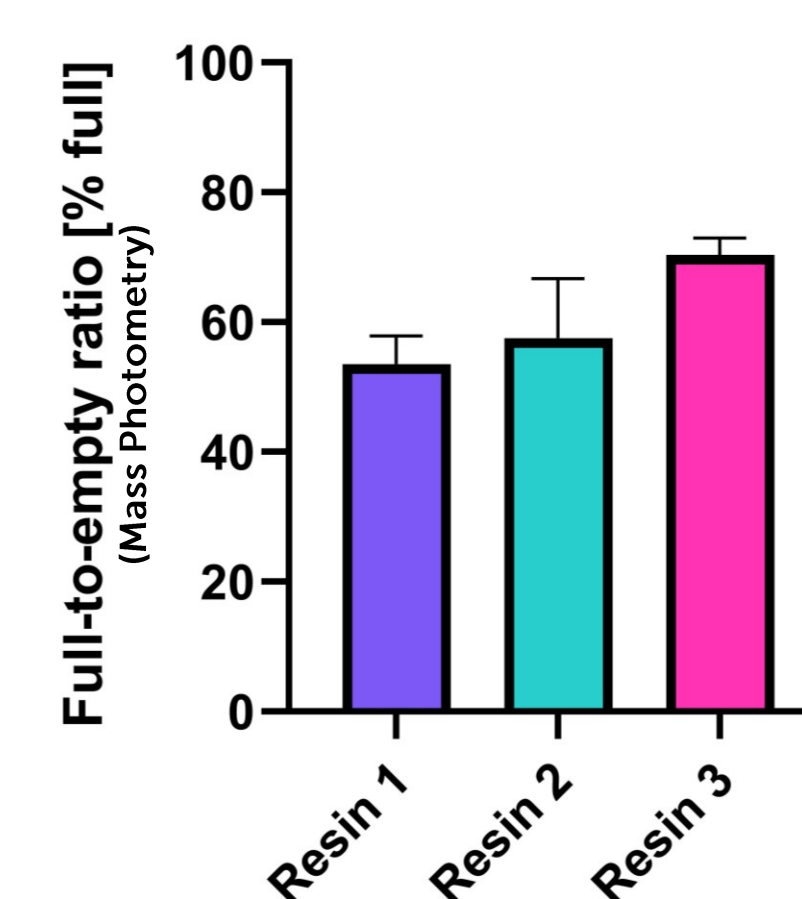
Characteristics of AEX Conditions

- **Loaded Material:**
 - Vector genome concentration: 1.0E+13 vg/mL (Droplet Digital PCR)
 - Full-to-empty ratio: 32% full (Mass Photometry)
 - Host cell protein impurities: 27 ng/mL (Gyrolab Immunoassay)
 - Total dsDNA impurities: 154 ng/mL (Qubit)
- **Anion Exchange Chromatography:**
 - Optimal buffer composition and AEX conditions for each resin (linear gradient)
 - Same total capsid concentration loading for all resins
 - Same fractionation scheme of eluates for all resins (conditional fractionation, UV_{260/280} > 1.20)

➤ Vector Genome Recovery:

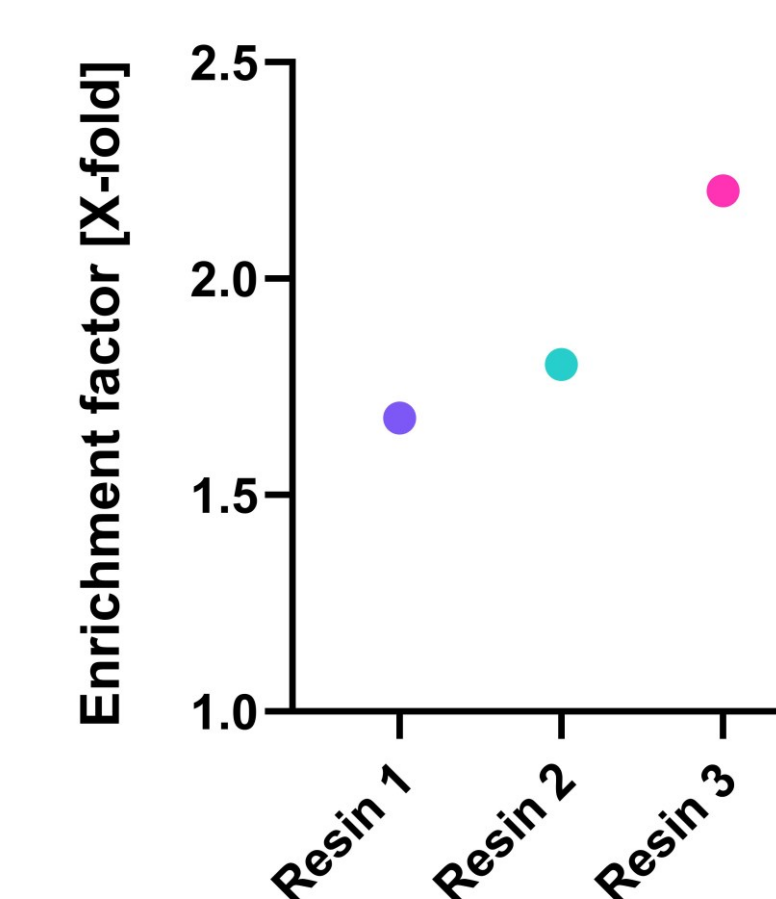


➤ Full-to-Empty Ratio:



Characteristics of AEX Eluates

➤ Full-to-Empty Enrichment:



➤ Impurities and Polydispersity (all Resins):

- Host cell protein impurities: < 4 ng/mL (LLOQ*) (Gyrolab Immunoassay)
- Total dsDNA impurities: < 50 ng/mL (LLOQ*) (Qubit)
- Polydispersity index: < 0.1 (Unchained Labs Stunner)

* lower limit of quantification

Conclusion All three resins showed similar performance in AAV8 full-to-empty capsid separation, with minor but relevant differences in resolution, vector genome recovery, and % full enrichment. All resins yielded high-quality eluates with low polydispersity and impurity levels. One resin achieved the best balance of quality and yield, with >70 % full capsids and 74 % vector genome recovery. Although the results reflect strong process performance, there remains potential to further refine yield and product quality.

Our purification platform is designed for flexibility, enabling adaptation of resin and process parameters to meet client-specific requirements regarding % full and step recovery. This adaptability, along with the ongoing development of GMP-ready steps to facilitate an efficient transfer into our GMP manufacturing facilities in the U.S., supports tailored solutions for partners seeking high-quality AAV8 material, whether for early- or late-stage development.

