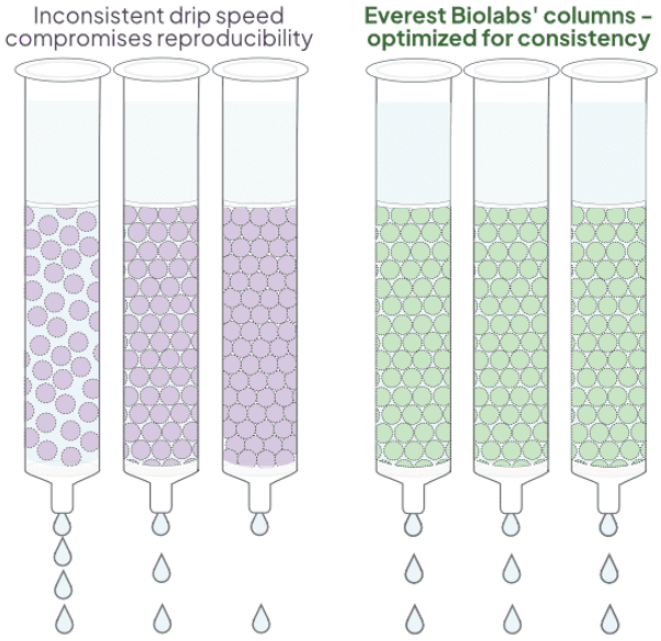
Drip speed matters: Enhance EV isolation reproducibility with Apex columns and validate using the Atlas ELISA

Column-to-column variability in extracellular vesicle (EV) isolation using size exclusion chromatography (SEC) can significantly impact downstream results—shifting the EV elution profile and compromising reproducibility. Here, we explore how drip speed influences EV yield and fractionation, and how Everest Biolabs' manufacturing standards—including drip-speed QC and EV profiling with the Atlas EV ELISA—ensure consistent and reliable EV isolation.



Drip speed serves as a proxy for column packing quality and resin flow behavior, both of which are critical for reproducible EV separation. Flow resistance in the resin bed affects retention time, diffusion, and shear forces (1). Differences in drip speed between columns can shift EV-containing fractions, alter yield, and introduce inconsistencies between experiments.

To illustrate how column packing affects drip speed and EV elution, we packed SEC columns with incrementally increasing Sepharose 6B resin volumes, compressing

each to the same final column height. This design increased the compression percentage at each step, resulting in:

- Slower drip speeds due to tighter resin packing and increased flow resistance (Figure 1A)
- Reduced void volume, leading to earlier EV elution in the fractionation profile (Figure 1B)

Evaluate EV elution and purity using Atlas ELISA

To assess how these differences impact EV recovery, we fractionated 0.5 mL of plasma using each column and collected 0.5 mL fractions. EV content in each fraction was quantified using the Everest Atlas EV ELISA—a fast, reliable, and direct assay for EV detection from biofluids or SEC fractions.

The assay involves a single incubation step with a cocktail of antibodies targeting the tetraspanins (CD9, CD63, and CD81), enabling robust, reproducible quantification of total EV levels. In addition, the Atlas HSA ELISA was used to quantify the amount of human serum albumin in each fraction. Together, these assays provide a streamlined way to evaluate isolation performance by tracking both EV recovery and protein contamination, using HSA as a representative marker for free plasma proteins.

Drip speed significantly affects EV elution profile

By measuring individual fractions with the Atlas EV ELISA, we observed a clear shift in the EV elution profile, which was dependent on the column drip speed. Columns with slower drip speeds—due to tighter resin compression—consistently produced earlier EV peaks, with the majority of EVs eluting in fractions 1–2. In contrast, faster-flowing columns exhibited a delayed elution profile, with the majority of EVs eluting in fractions 2–3 (Figure 1B, 1C).

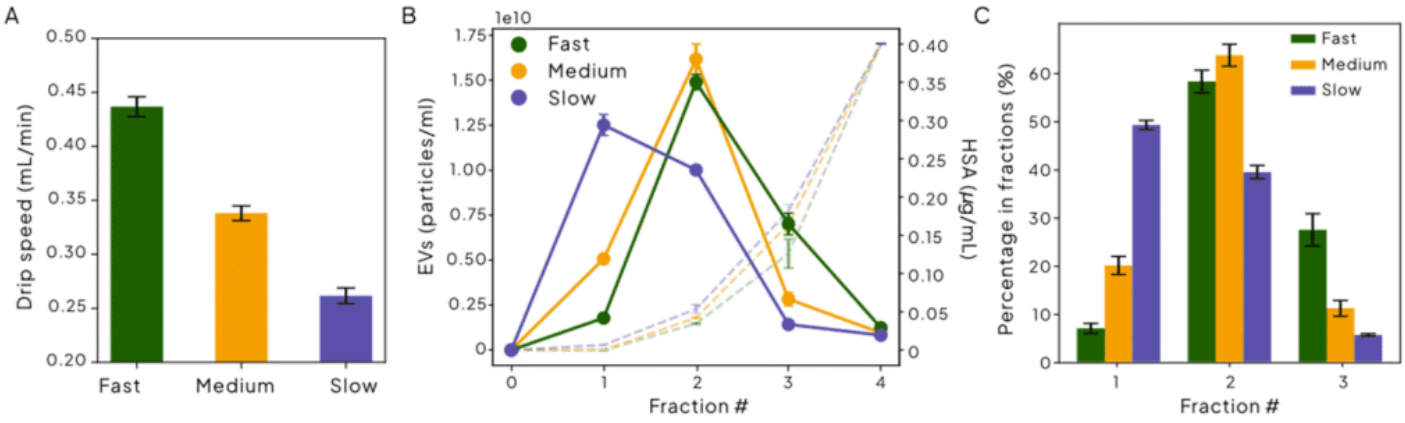


Figure 1. Characterization of SEC columns with different drip speeds. (A) Drip speed was measured for each column type after equilibration. (B) EV elution profiles (solid lines) and HSA elution profiles (dashed lines) show differences in fractionation across columns with varying drip speeds. (C) Percentage of total EVs recovered in each fraction, highlighting the shift in EV elution profiles associated with different column drip speeds.

Pooled EV fractions will recover yield but result in variable purity

Typically, large studies pool EV-containing fractions (1-3) across all samples. Variability in drip speed between columns can lead to inconsistent EV yields and purities from those fractions (Table 1) leading to higher CVs across the study.

	Relative yield (fractions 1-3)	Relative purity (fractions 1-3)
Fast	0.95±0.01	0.85±0.03
Medium	0.98±0.02	0.92±0.02
Slow	0.94±0.03	0.98±0.02

Apex columns are QC’d for reproducible EV elution and purity

To address this, we optimized our Apex SEC columns to deliver a reproducible EV elution profile by validating each lot for consistent drip speed (Figure 2) and verifying elution by Atlas EV ELISA. Pairing the Apex columns with an automated, high-throughput fraction collector like the Ascent instrument further minimizes variability in EV elution across columns.

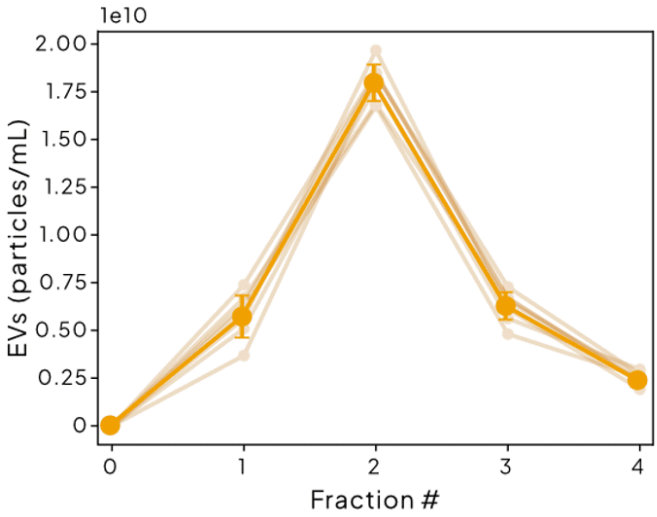


Figure 2: EV elution profile of 8 Apex 6B columns optimized for consistent drip speed. CV (EV yield) =4.3%

Reference:

1. Jonathan J. Stickel, Alexandros Fotopoulos, Pressure-Flow Relationships for Packed Beds of Compressible Chromatography Media at Laboratory and Production Scale, 2008