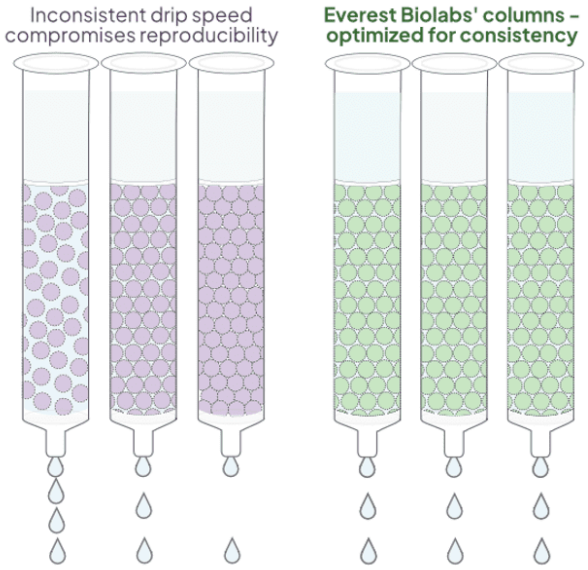
APEX · QUALITY CONTROL

Drip speed matters: enhancing EV isolation reproducibility with Apex columns

Apex™ SEC columns are for research use only. Not for use in diagnostic procedures.

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, reliable EV isolation.

Drip speed serves as a proxy for column packing quality and resin flow behavior, both 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.



Variable column packing (left) shifts the EV elution profile between columns; Apex columns QC'd for consistent drip speed (right) deliver reproducible isolation.

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).

EVALUATING ELUTION & PURITY WITH 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, direct assay for EV detection from biofluids or SEC fractions.

The assay uses a single incubation with a cocktail of antibodies targeting the tetraspanins (CD9, CD63, CD81), enabling robust, reproducible quantification of total EV levels. The Atlas HSA ELISA was also used to quantify human serum albumin per fraction, tracking both EV recovery and protein contamination (HSA as a marker for free plasma proteins).

DRIP SPEED SHIFTS THE EV ELUTION PROFILE

By measuring individual fractions with the Atlas EV ELISA, we observed a clear shift in the EV elution profile that depended on column drip speed. Columns with slower drip speeds, due to tighter resin compression, consistently produced earlier EV peaks, with most EVs eluting in fractions 1–2. Faster-flowing columns showed a delayed profile, with most EVs eluting in fractions 2–3 (Figure 1B, 1C).

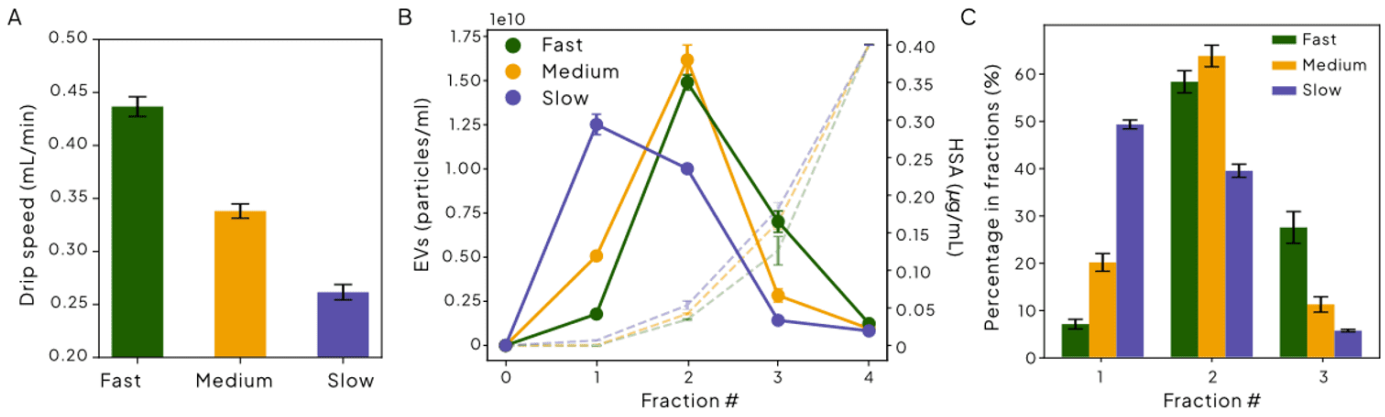


Figure 1. Characterization of SEC columns with different drip speeds. (A) Drip speed measured for each column type after equilibration. (B) EV elution profiles (solid lines) and HSA elution profiles (dashed lines) across columns with varying drip speeds. (C) Percentage of total EVs recovered in each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

POOLING FRACTIONS: YIELD VS. 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), driving higher CVs across a study.

Drip speed	Rel. yield (fx 1–3)	Rel. purity (fx 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

Table 1. *Relative EV yield and purity from pooled fractions 1–3 for fast, medium, and slow columns (mean ± SD).*

APEX COLUMNS ARE QC'D FOR REPRODUCIBILITY

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 Apex columns with an automated, high-throughput fraction collector like the Ascent instrument further minimizes variability in EV elution across columns.

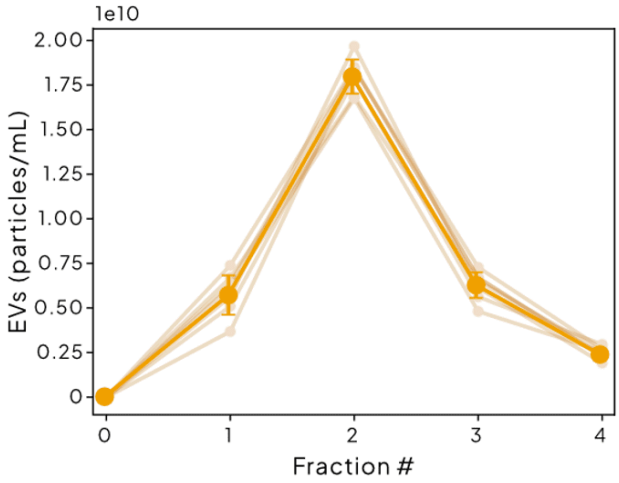


Figure 2. Lot-to-lot reproducibility. EV elution profiles of 8 Apex 6B columns optimized for consistent drip speed; CV of EV yield = 4.3%.

CONCLUSION

Drip speed is a measurable proxy for packing quality that directly shifts the EV elution profile. By QC-ing every Apex lot for consistent drip speed and verifying elution by Atlas EV ELISA, Everest delivers reproducible EV recovery, and pairing Apex with the Ascent removes operator-dependent variability entirely.

REFERENCE

1. Stickel JJ, Fotopoulos A. Pressure-flow relationships for packed beds of compressible chromatography media at laboratory and production scale. *Biotechnol Prog*. 2008.