APEX · SEC COLUMNS

Can SEC be a scalable and more reproducible alternative to ultracentrifugation?

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

EV labs are moving away from the rotor toward size-exclusion chromatography (SEC). The reason: SEC preserves EV biophysical integrity and functionality more effectively than ultracentrifugation (UC), which subjects vesicles to high gravitational forces that can cause aggregation, membrane damage, and reduced biological activity.[1–4]

In the table below, we compare SEC to differential ultracentrifugation (DUC) and density-gradient ultracentrifugation (DGUC).[1–6] SEC provides a good balance between yield, purity, and scalability without expensive instrumentation, making it accessible to most labs. DGUC sets the standard for purity; however, we show that SEC can be improved to address the drawbacks of size-only separation.

METHOD COMPARISON

Parameter	Differential UC	Density-gradient UC	Size-exclusion (SEC)
Separation principle	Sedimentation by size / density	Buoyant density in gradient medium	Hydrodynamic radius (size)
EV yield	Low–moderate	Low	High
Protein removal (purity)	Low–moderate	High	Moderate–high
Lipoprotein depletion	Poor	High (low-density lipoproteins)	Poor (SEC); high (multi-mode)
EV integrity / function	Reduced (aggregation risk)	Moderate (long processing)	High
Processing time / run	4–18 hours	16–72 hours	~30 minutes
Scalability / throughput	Low	Low	High (parallelizable, automatable)
Equipment	Ultracentrifuge	Ultracentrifuge	SEC column; automation optional

Table 1. *Comparison of EV isolation methods across separation principle, yield, purity, integrity, throughput, and equipment.*

SEC WITH APEX COLUMNS

The Apex SEC columns are optimized to deliver a reproducible EV elution profile by validating each lot for consistent drip speed and verifying elution by Atlas EV ELISA. Apex 6B columns are packed with Sepharose CL-6B and optimized for the highest EV recovery, ideal for separating EVs from soluble proteins in less complex samples such as cell culture, CSF, and urine. For demanding samples like plasma and serum, Apex 4B columns are packed with Sepharose CL-4B and optimized for the highest purity of secreted proteins.

The lipoprotein challenge & Apex MM

MISEV2023 highlights that no single separation method fully resolves EVs from co-isolating non-vesicular extracellular particles (NVEPs), and recommends that researchers select and combine methods based on the requirements of their downstream application.[5] Separating EVs from NVEPs is most challenging when isolating from serum or plasma, where lipoproteins are several orders of magnitude more abundant than EVs. These difficulties are present in both UC and SEC, except when using laborious density-gradient UC.

Apex MM columns address this challenge by combining SEC resin of small pore size with a multi-mode (MM) resin (Figure 1). This resin uses hydrophobic interactions and ion-exchange to selectively bind and remove lipoproteins and other soluble contaminants. Apex MM columns are particularly advantageous for unbiased downstream analyses, such as proteomics, where purity is paramount.

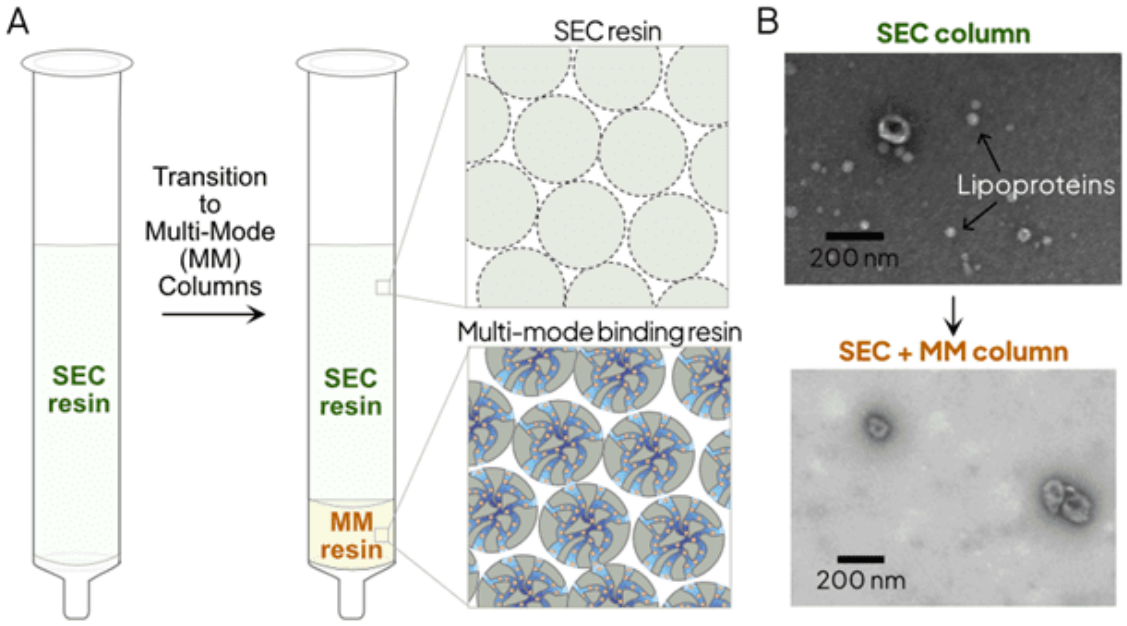

Figure 1. Apex MM column architecture. (A) Transition from standard SEC to multi-mode chromatography; the integrated MM resin layer provides a secondary purification step by binding lipoproteins and soluble proteins through hydrophobic and ionic interactions. (B) TEM images of EVs isolated using a standard SEC column (top) and an Apex MM column (bottom).

AUTOMATION FOR SCALABLE PROCESSING

The Ascent instrument enables semi-automated SEC-based EV isolation for up to 8 samples simultaneously, while the Summit instrument scales fully automated isolation from 1 up to 48 samples at a time. By eliminating operator-dependent variability, these instruments support the throughput required for biomarker discovery and clinical research, addressing the key historical limitation of manual SEC.

Concentrated recovery

For applications requiring low input volumes, SEC fractionation can be coupled with ultrafiltration to deliver concentrated EV fractions. On the Ascent instrument this is achieved by collecting EVs directly into centrifugal concentrators; the Summit instrument integrates SEC isolation and ultrafiltration in a fully automated, plate-based format for up to 48 samples in parallel.

SUMMARY

- SEC preserves EV integrity and functionality where ultracentrifugation risks aggregation and membrane damage.
- Apex MM columns resolve the lipoprotein co-isolation problem, achieving DGUC-level purity in an automatable workflow.
- Paired with the Ascent and Summit platforms, SEC becomes standardized and high-throughput, no longer expert-only.

REFERENCES

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