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Application Note

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Combining Sequential Multi-Column Chromatography With Sartobind® Rapid A: A Proof of Concept From Process Development to Pilot Scale

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Abstract

Sequential multi-column chromatography (S-MCC) is often evaluated using Protein A resins for antibody purification, but has rarely been tested using membranes. This proof-of-concept study tested the use of Sartobind® Rapid A membranes in S-MCC for a monoclonal antibody capture application.

The process was first developed on Resolute® BioSMB PD before being scaled up on a custom Resolute® BioSC Pilot system, using 75 mL Sartobind® Rapid A capsules. Despite some necessary adjustments to adapt the process to the pilot-scale system, we demonstrated similar yield (> 97%) and impurity clearance when targeting 47 g/L loading (100% of the dynamic binding capacity at 10%).

Our conclusion is that S-MCC enables 20% higher loading than standard rapid cycling chromatography, while also reducing buffer consumption by the same proportion.

Introduction

Monoclonal antibody (mAb) capture remains one of the largest cost drivers in mAb production, particularly during clinical trials. At this stage, companies often spend hundreds of thousands on costly affinity resins that are typically used only 1–5 times before being discarded. Resins also require extra hardware, such as columns and packing skids, which bring additional costs, while ordering prepacked columns increases both cost and lead time. Packed resins must then be stored for extended periods between clinical batches, which results in added costs and the need for cleaning and lifetime validation. Beyond the resin itself, process development and validation for the affinity step alone take 2–3 weeks in Phase 1 and extend to 3–4 months in Phase 3.

Membrane technology addresses these bottlenecks by significantly reducing run times, enabling process development and validation within 7 and 20 working days for Phases 1 and 3, respectively. Rapid cycling chromatography (RCC) further reduces media volume and lowers capture costs by more than 40% during clinical trials. The ease of use inherent to the prepacked format is also a key advantage, minimizing low-value-added operations and enabling the production of more batches in multi-product facilities.

However, one of the current drawbacks of membrane chromatography is its higher buffer consumption. In this study, we investigated the use of sequential multi-column chromatography (S-MCC) to increase the loading per cycle and reduce the buffer consumption. The study was carried out in collaboration between LFB Biomédicaments (Les Ulis, France), LFB Biomanufacturing (its contract development and manufacturing organization in Alès, France), and Sartorius (Göttingen, Germany). The process was developed in Alès and Göttingen using Resolute® BioSMB PD with Sartobind® Rapid A Nano 1.2 mL membranes. Scale-up runs were then performed on a custom Resolute® BioSC Pilot system in Les Ulis, using 75 mL Sartobind® Rapid A capsules. Here, we demonstrate that this strategy works well for Sartobind® Rapid A and can scale up successfully.

Methods

The laboratory-scale trials were conducted using Resolute® BioSMB PD with three Sartobind® Rapid A 1.2 mL Nano membranes. The scale-up tests were performed with a Resolute® BioSC Pilot chromatography system (Figure 1), using three 75 mL Sartobind® Rapid A capsules with a column volume (CV) of 75 mL.

The clarified harvest used at small scale had a mAb concentration of 2.92 g/L after filtration on a 0.2 µm PES membrane. The clarified harvest used at pilot scale had a concentration of 2.54 g/L after filtration on Sartoclean® (0.8–0.65 µm; 1.8 m^2) and Sartopore® 2 (0.45–0.2 µm; 1.8 m^2) filters. The concentrations likely differ due to variations in storage procedures, freezing | thawing cycles and additional filtrations. Both concentrations were assessed by Protein A high-performance liquid chromatography (HPLC). Table 1 lists the buffers used in this study.

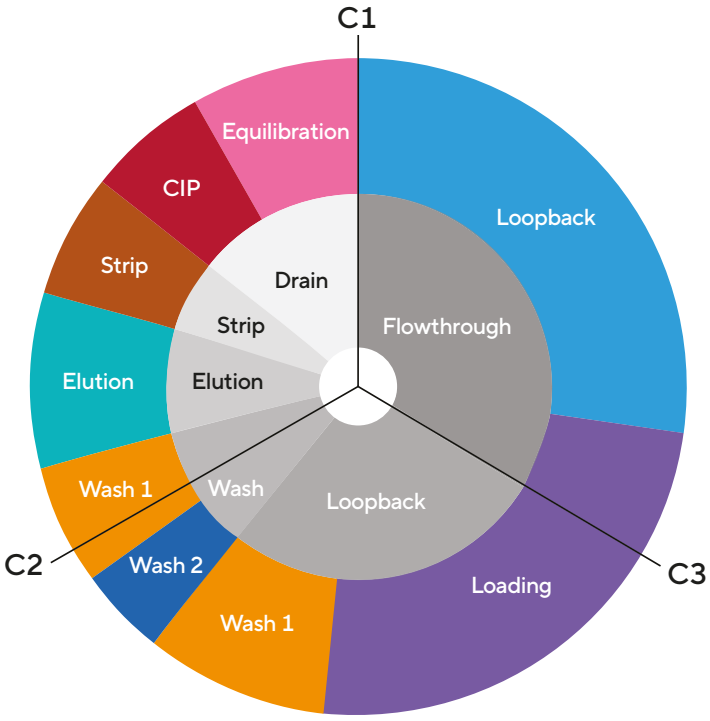
Table 1: List of buffers used for S-MCC using Sartobind® Rapid A

Step	Buffer
Equilibration	25 mM Tris, 5 mM EDTA, 25 mM NaCl, pH 7.1
Loopback	Flowthrough from column being loaded
Load	Clarified harvest
Wash 1	25 mM Tris, 5 mM EDTA, 25 mM NaCl, pH 7.1
Wash 2	25 mM Tris, 5 mM EDTA, 1.2 M NaCl, pH 7.1
Wash 3	25 mM Tris, 5 mM EDTA, 25 mM NaCl, pH 7.1
Elution	100 mM acetate, pH 3.5
Strip	100 mM sodium acetate, 1 M NaCl, pH 2.9
CIP	NaOH 0.2 M

Figure 1: Custom Resolute® BioSC pilot system with three 75 mL Sartobind® Rapid A capsules

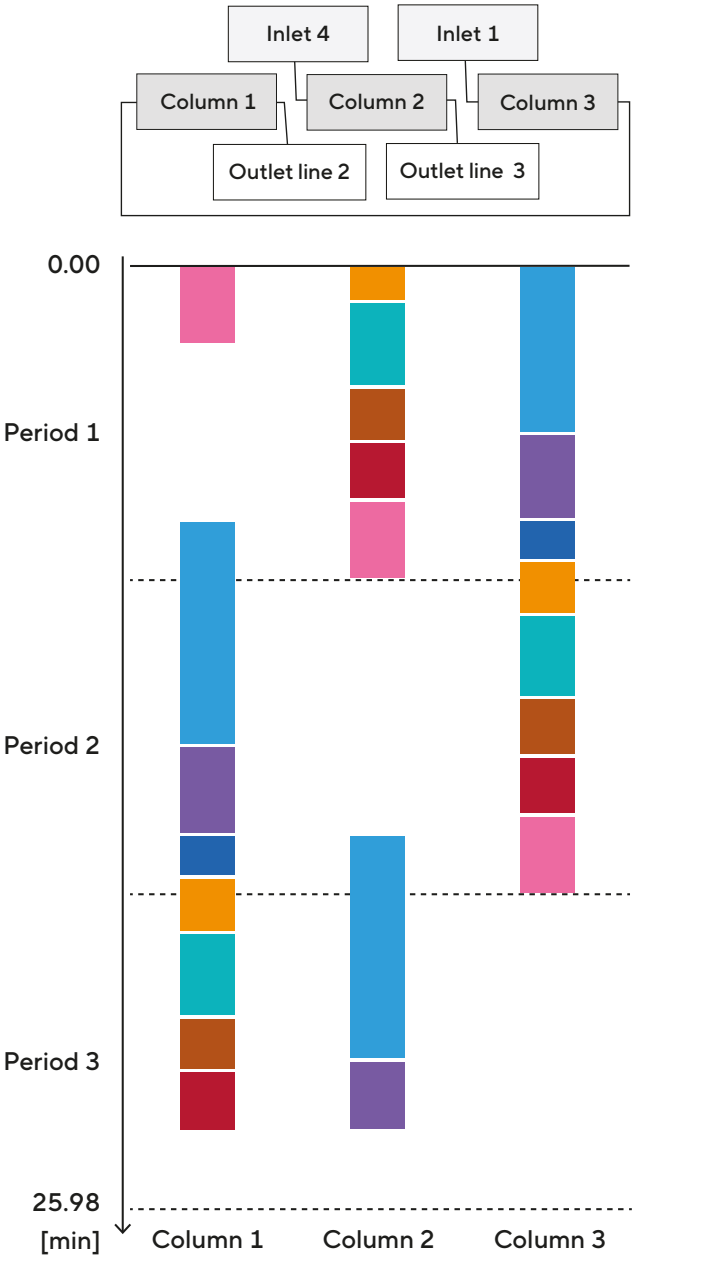
Table 2 shows the recipe followed at both scales. The methods used on both systems are shown in Figures 2 and 3. The transition from Resolute® BioSMB PD software to Resolute® BioSC Pilot was simple and effective: only a minor adjustment was necessary to adapt the wash volumes.

Figure 2: Resolute® BioSMB PD recipe



Note. As the membranes run clockwise, the outer circle corresponds to the inlet, and the inner circle to the outlet. The loopback inlet | outlet allows column connections.

Figure 3: Resolute® BioSC Pilot recipe



Note. The recipe is translated from Figure 2 into Resolute® BioSC software. Colored bars represent different steps: orange (wash 1), dark blue (wash 2), grey (wash 3), teal (elution), brown (strip), red (CIP), pink (equilibration). When no block or no color is mentioned (white), the column is connected with column n-1.

Table 2: Recipe used for S-MCC using Sartobind® Rapid A

Step	Volume [CV]		Flow rate [CV/min]	
	Lab scale	Pilot scale	Lab scale	Pilot scale
Equilibration	15	15	6.9	6.9
Load	19*	19*	3.0	3.0
Wash 1	12**	7**	3.0	3.0
Wash 2	8	8	5.2	6.9
Wash 3	10	10	7.1	6.4
Elution	15	15	6.7	6.7
Strip	10	10	6.4	6.4
CIP	10	10	6.4	6.4

* The target was to load 47 g/L. The loading was the same in both scales, as the starting material was from the same batch. Due to differences in clarified harvest concentrations, loading was performed at 55 g/L at lab scale and at 47 g/L at pilot scale.

** Wash 1 volume had to be adapted to the system design.

Results

Before conducting the S-MCC experiments, Sartobind® Rapid A was assessed as a standard batch chromatography medium at laboratory scale. The dynamic binding capacity at 10% (DBC10%) was measured at 47 g/L (data not shown).

Resolute® BioSMB PD

Various tests were performed to define the loading in S-MCC mode, up to 60 g/L. Above 55 g/L, we observed a yield reduction due to membrane saturation and product leakage in the flowthrough. Therefore, we decided to target 47 g/L loading to maintain a reasonable safety margin at all scales. However, for the small-scale test, the titer evaluated by Protein A HPLC was higher than expected (2.92 g/L rather than 2.54 g/L), which resulted in membrane overloading to 55 g/L.

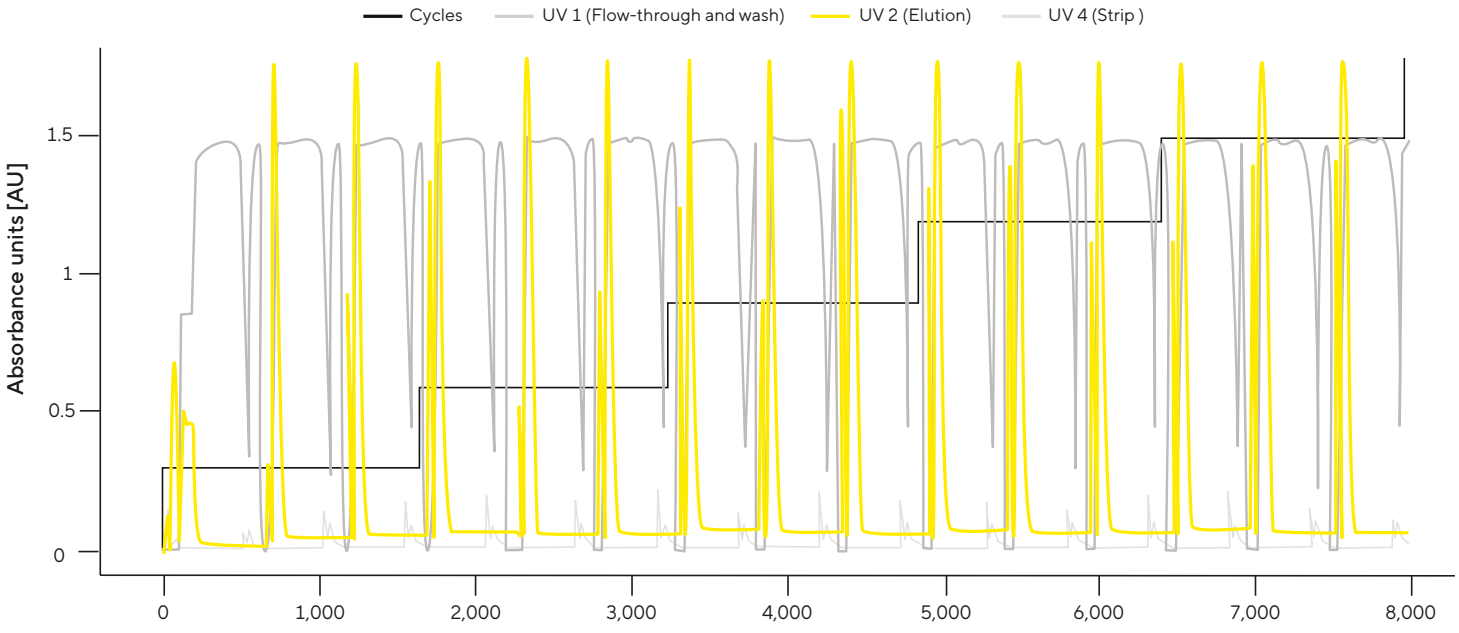
During the lab-scale test, five cycles were performed, plus one elution cycle. The results are summarized in Table 3 and the chromatogram is shown in Figure 4. Each cycle required 26 minutes. A total of 999 mg was loaded over 2 hours and 9 minutes, and 977 mg were found in the eluate pool, resulting in a 98% yield despite the overloading.

Table 3: S-MCC results using Sartobind® Rapid A at small scale

Monomer % in eluate	HMW % in eluate	HCP LRV	DNA [µg/g]	Yield [%]
95.52	3.88	1.82	1.88	98%

Note. HMW = high molecular weight, HCP = host cell protein, LRV = log reduction value

Figure 4: Chromatogram from the small-scale test on Resolute® BioSMB PD



Resolute® BioSC Pilot

The scale-up was conducted in another facility (Les Ulis, France), using the clarified harvest from the same batch. The product used as a starting material was slightly different due to filtration treatment, with an actual loading titer of 2.54 g/L. Therefore, the actual loading of the membrane matched the target (46.5 g/L vs. 47.0 g/L targeted). Eight cycles were performed on the three 75 mL membranes within 3 hours and 28 minutes. The cycle time remained consistent with small scale runs (26 minutes), and minor adjustments had to be made on the recipe to adapt to the system pump (Table 2). Results are summarized in Table 4 and Figure 5.

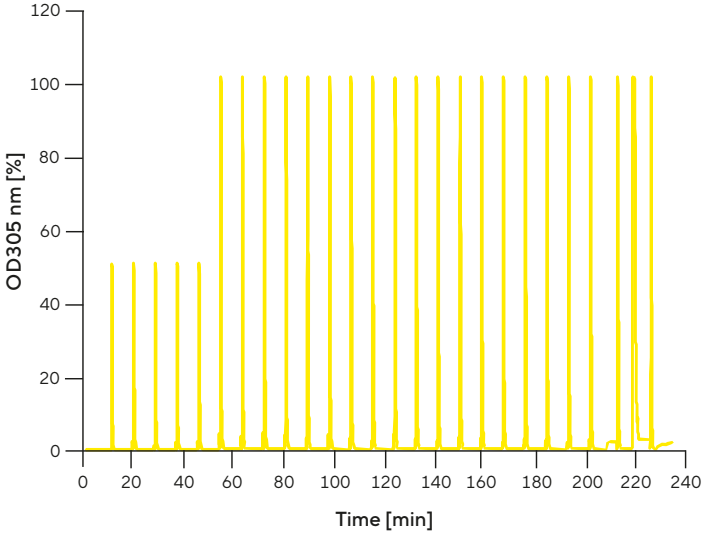
Table 4: S-MCC results using Sartobind® Rapid A at pilot scale

Monomer % in eluate	HMW % in eluate	HCP LRV	DNA [µg/g]	Yield [%]
95.23	4.51	1.62 to 1.82*	0.87	97%

Note. HMW = high molecular weight, HCP = host cell protein, LRV = log reduction value

* Depending on whether we consider the HCP in the load or in the sum of flowthrough fractions

Figure 5: Chromatogram from the pilot-scale test on custom Resolute® BioSC (elution line)



Note. Absorbance was measured at 305 nm. The signal range was adjusted after the fifth cycle, which caused a change in the peak height from cycle six to the end of the experiment.

Discussion

This proof-of-concept study was performed to demonstrate that an S-MCC approach with Sartobind® Rapid A membranes can be successfully scaled up from Resolute® BioSMB PD to Resolute® BioSC. We demonstrated similar yield and purity at both scales, even when overloading the Sartobind® Rapid A Nano membranes on the Resolute® BioSMB PD system.

The next question is whether using S-MCC with Sartobind® Rapid A is relevant: Does it bring additional benefits compared to RCC?

Table 5 compares RCC and S-MCC approaches for this specific case from the perspective of productivity and buffer requirements. The increased loading per cycle in S-MCC compared to RCC results in a 20% reduction in buffer consumption. However, the loading flow rate usually decreases when membranes are used in S-MCC mode. In fact, membrane thickness remains the same as that used in batch mode, and connecting them in series leads to a higher pressure drop.

Table 5: Comparison of RCC and S-MCC using Sartobind® Rapid A

Evaluation criteria	RCC	S-MCC
Loading [g/L]	37.6	47.0
Number of membranes	1	3
Buffer consumption [L/g]	2.13	1.70
Productivity [g/L/h]	193	108

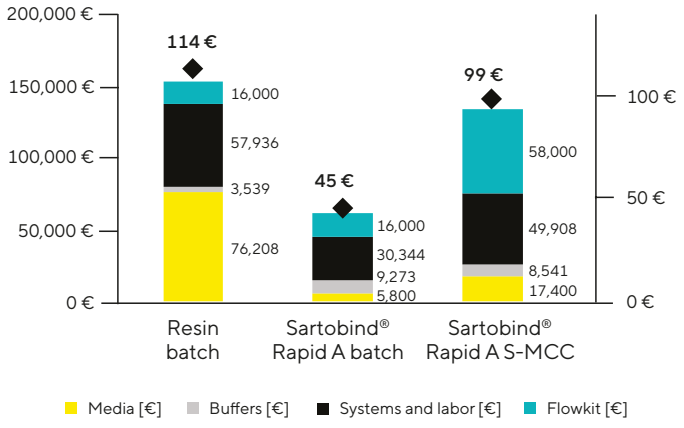
Note. Results were calculated for 50 cycles in both cases

Buffer savings could be further increased with higher loading (for example, 105 – 110% of the DBC10% rather than 100%). Since we observed a breakthrough from 55 g/L (117% of DBC10%), the target loading should stay below that value. Nevertheless, defining the optimum loading would require a thorough assessment to ensure enough safety margin in terms of product recovery, considering process and consumable variability.

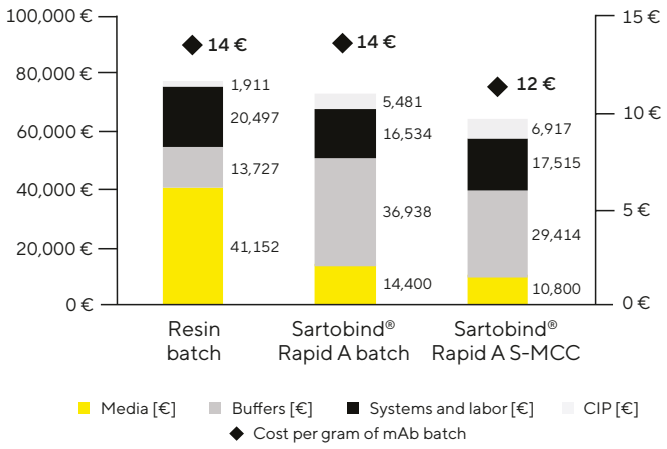
Finally, the most important benefit lies in the cost of goods: buffer reduction, combined with improved membrane utilization, can lower production costs across a range of cases. However, cost savings strongly depend on the number of batches per year, the antibody titer, and the choice between single- or multi-use approaches. A general trend we observe is that RCC is easier to implement and more cost-effective for small numbers of batches, whereas S-MCC offers cost advantages at commercial production scale, as shown in Figure 6.

Figure 6: Process projections at clinical and commercial scale

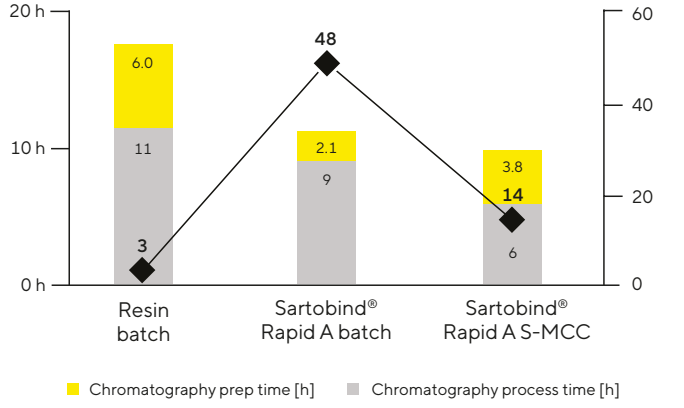
Case 1: Clinical production: Costs per batch



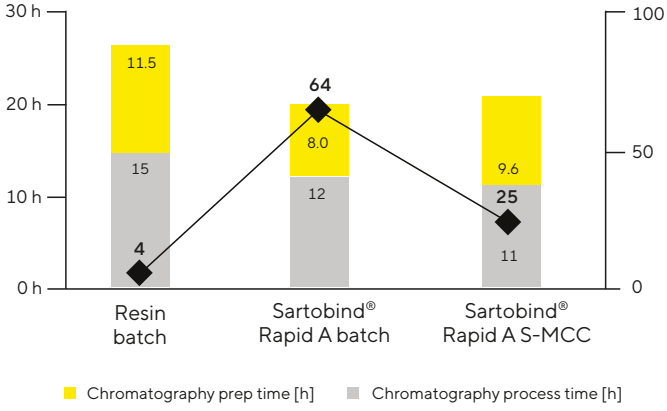
Case 2: Commercial production: Costs per batch



Case 1: Clinical production: Process time and number of cycles



Case 2: Commercial production: Process time and number of cycles



Note. Case 1: Clinical production scenario: 500 L batch at 3 g/L, 2 batches per year, on a single-use chromatography system. Loading of 48 g/L for the resin, 37 g/L for Sartobind® Rapid A in batch (RCC) mode, and 47 g/L for Sartobind® Rapid A in S-MCC mode.

Case 2: Commercial production scenario: 2,000 L batch at 3 g/L, 12 batches per year, on a stainless-steel chromatography system. Loading of 45 g/L for the resin, 37 g/L for Sartobind® Rapid A in batch (RCC) mode, and 50 g/L (107% of DBC10% was assumed to encompass sufficient safety margin) for Sartobind® Rapid A in S-MCC mode.

For both cases, the projections were performed using the same recipe and buffers as in this study, and the resin loading was adjusted depending on the standard column size and total mAb mass to process divided by the number of cycles, while keeping bed height constant.

Conclusion

In conclusion, S-MCC (the sequential multi-column chromatography concept carried out in this case with chromatographic membranes) has been demonstrated to scale up successfully with Sartobind® Rapid A. S-MCC can bring significant cost benefits, and its implementation should be discussed on a case-by-case basis, like most process intensification technologies.

Sartorius has developed the Expert Chromatography Intensifier Tool (ExCIT), which was used in this study. It is designed to predict and select the best process intensification technologies for each specific production scenario and can support biopharmaceutical producers on their process intensification journey.

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