From a dead forgotten innovation to a five-star product: How slalom chromatography found new purpose in cell and gene therapy

Slalom chromatography (SC) is a separation technique originally discovered in 1988 [1,2] and abandoned more than 25 years ago for the lack of proper understanding of its separation mechanism, speed, adequate columns and equipment, and large application fields. It was recently revitalized using bio-inert, ultra-high-pressure liquid chromatography (UHPLC) columns and systems for analyzing large DNA/RNA fragments from 3 kbp up to 30 kbp. The first commercial SC column (GT x Resolve™ Slalom Column, MaxPeak™ Premier 2.5 µm, 4.6 × 300 mm) became available in 2025 [3].

In this presentation, we revisit the research and development of the new column and highlight its principal applications. We begin by detailing the foundation of the SC separation mechanism, namely the slow coil-to-stretch transition exhibited by large biopolymers (e.g., dsDNA and dsRNA) under combined extensional and shear flow in the interparticle domain of UHPLC columns. In the second part, we report on the reproducibility of retention and efficiency in the SC column and provide a simple tool that enables straightforward method development in a design space involving flow rate, buffer concentration, and temperature to achieve maximum resolution of linear dsDNAs.

The third part of the presentation focuses on relevant applications in cell and gene therapy workflow, including plasmid purity assessment (purification of the desired supercoiled plasmid and removal of open-circular plasmid), plasmid linearization efficiency (separation of supercoiled and linear dsDNA) for in vitro transcription (IVT) of mRNA vaccines, dsRNA profile characterization in mRNA samples, and DNA restriction mapping. Performance comparison to gel electrophoresis will be shown. The extension of SC to the fractionation of synthetic or chemically modified natural polymers is also demonstrated for large hydroxyethyl cellulose polymers. Since the 30 cm long SC column is operated at high speed (~ 1 mL/min) and pressure drop (~ 10,000 psi), it is shown how its efficiency is improved by 25% by placing the column in a vacuum (10^{-5} mPa) chamber. Finally, the potential for semi-preparative SC will also be demonstrated using a tandem UHPLC–analytical fraction manager system.

Overall, this presentation introduces SC as a powerful bioanalytical technique for rapid, high-resolution characterization of large nucleic acids in plasmid and mRNA manufacturing workflows.

[1] J. Hirabayashi, K.-I. Kasai, Slalom chromatography: a new size-dependent separation method for DNA, Nucleic Acid Res. Symp. Ser., 20, 1988, 67-68.

[2] B. Boyes, D. Walker, P. McGeer, Separation of large DNA restriction fragments on a size-exclusion column by a nonideal mechanism, Anal. Biochem., 70, 1988, 127-134.

[3] F. Gritti, Retention mechanism in slalom chromatography: Perspectives on the characterization of large DNA and RNA biopolymers in cell and gene therapy, J. Chromatogr. A, 1743, 2025, 465691.