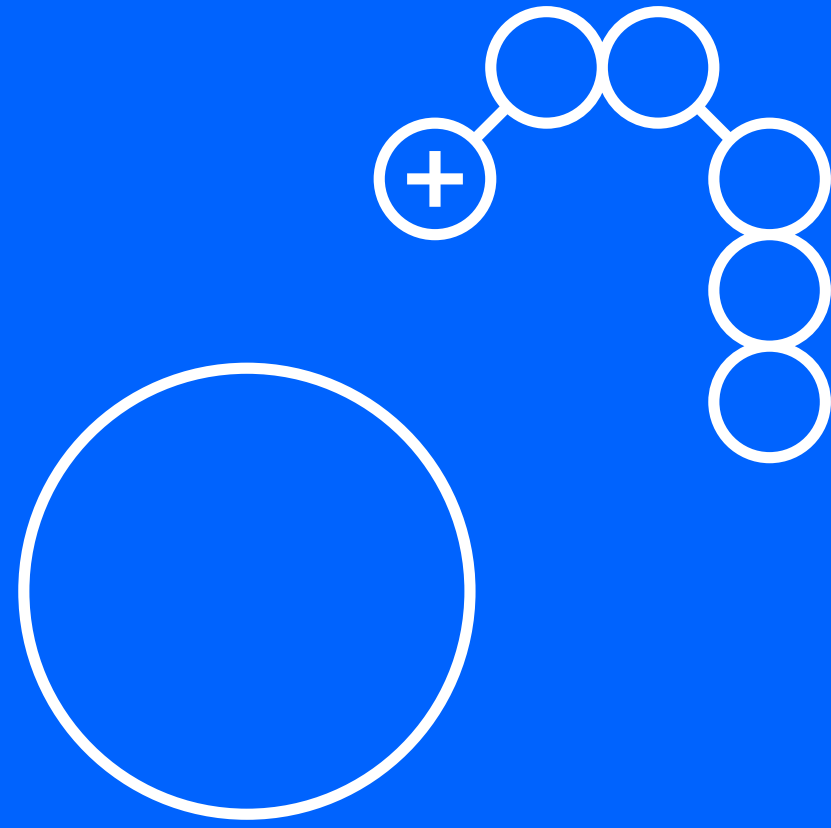


Exploring the Compatibility of Agarose-based AIEX Resin Columns with HPLC Systems

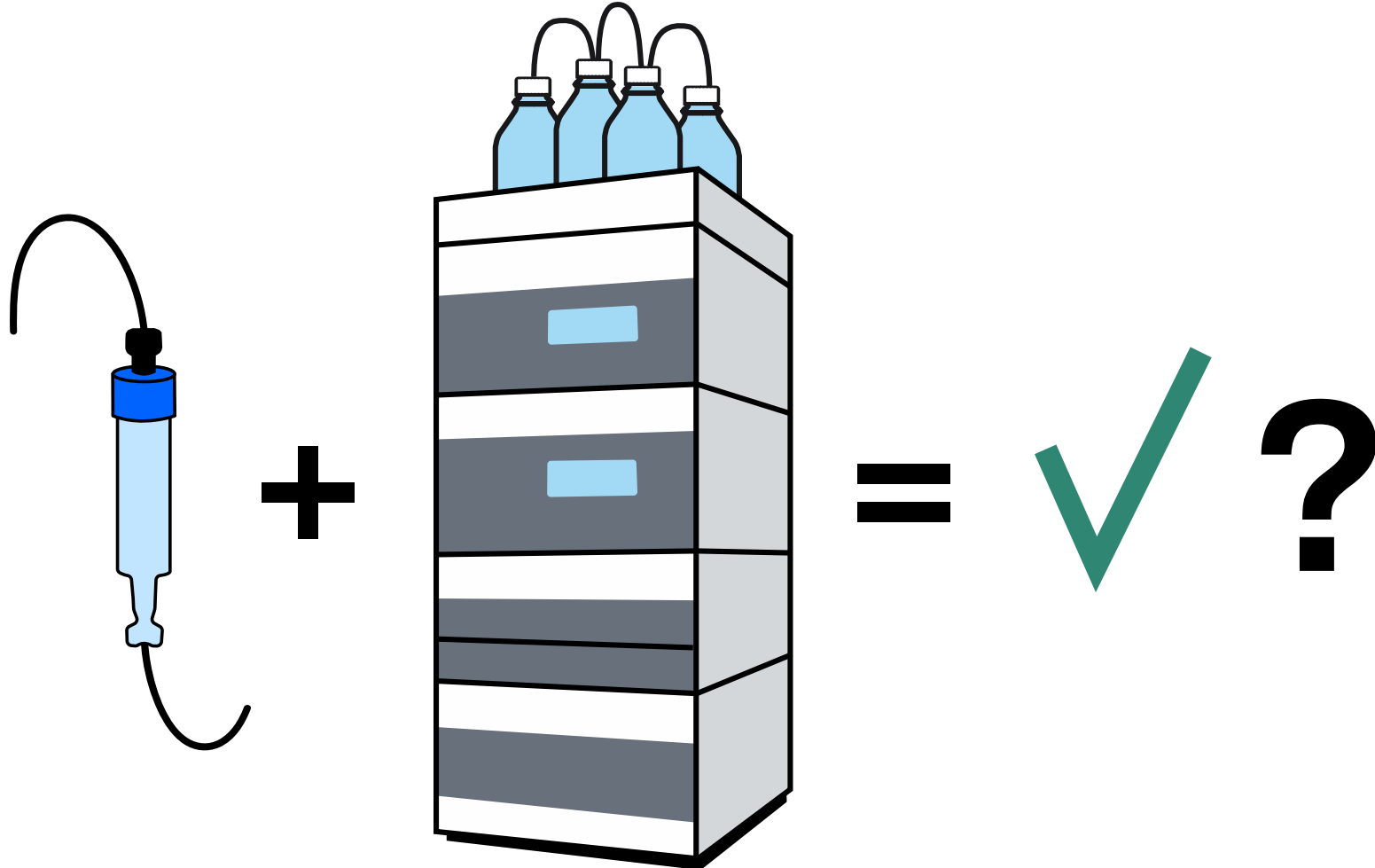
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Summary

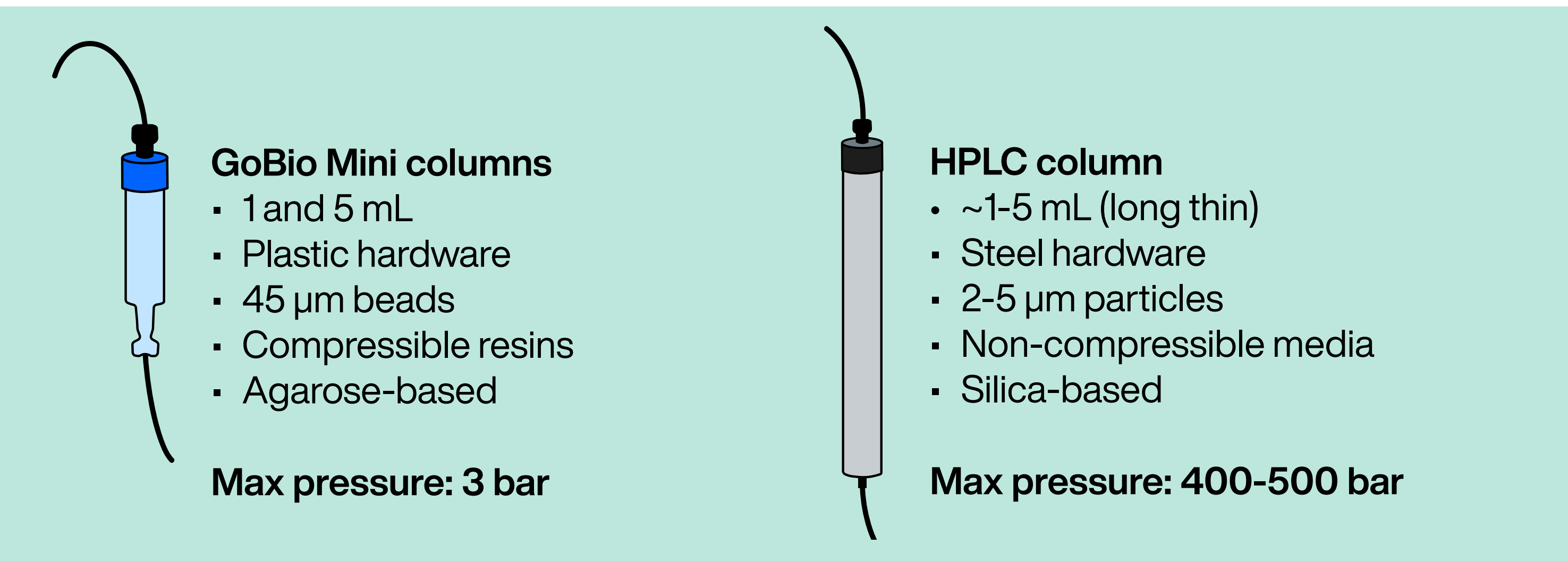
The increasing demand for sustainable and environmentally friendly purification techniques has led to a shift from traditional rigid media such as reversed-phase chromatography (RPC) and ion-pairing chromatography (IPC) to compressible resin-based methods, such as ion exchange chromatography (IEX). While rigid media are compatible with high-pressure liquid chromatography (HPLC) systems, compressible resins, such as agarose-based resins, traditionally require low-pressure liquid chromatography (LPLC) systems, like ÄKTA, due to their limited pressure tolerance.

However, system limitations, such as the exclusive availability of HPLC, can hinder this transition. If prepacked IEX resins could be ran on HPLC systems, this shift would be feasible, particularly at small scale. In this study, we demonstrate the compatibility of prepacked agarose-based resin columns with HPLC systems, using a DNA ladder of defined sizes as a proof-of-concept.



Pressure limitations

The main difference between an ÄKTA system and an HPLC systems is the system pressure. HPLC is traditionally used for high-resolution separations of small molecules and synthetic molecules (peptides and oligonucleotides) using small particle-sized RPC media generating high pressures, whereas an ÄKTA system operates at low pressures and has mainly been used for more preparative purposes to purify biomolecules.



For prepacked resins it is often the hardware that sets the pressure limits.

GoBio Minis have a max pressure of 3 bar. The agarose beads are much bigger than RPC particles, which means that the resins and columns themselves do not contribute to any substantial backpressures.

The column hardware pressure limit depends on the system pressure prior to the column, P_{pre} , see Figure 1.

It is important not to exceed the column hardware pressure limit.

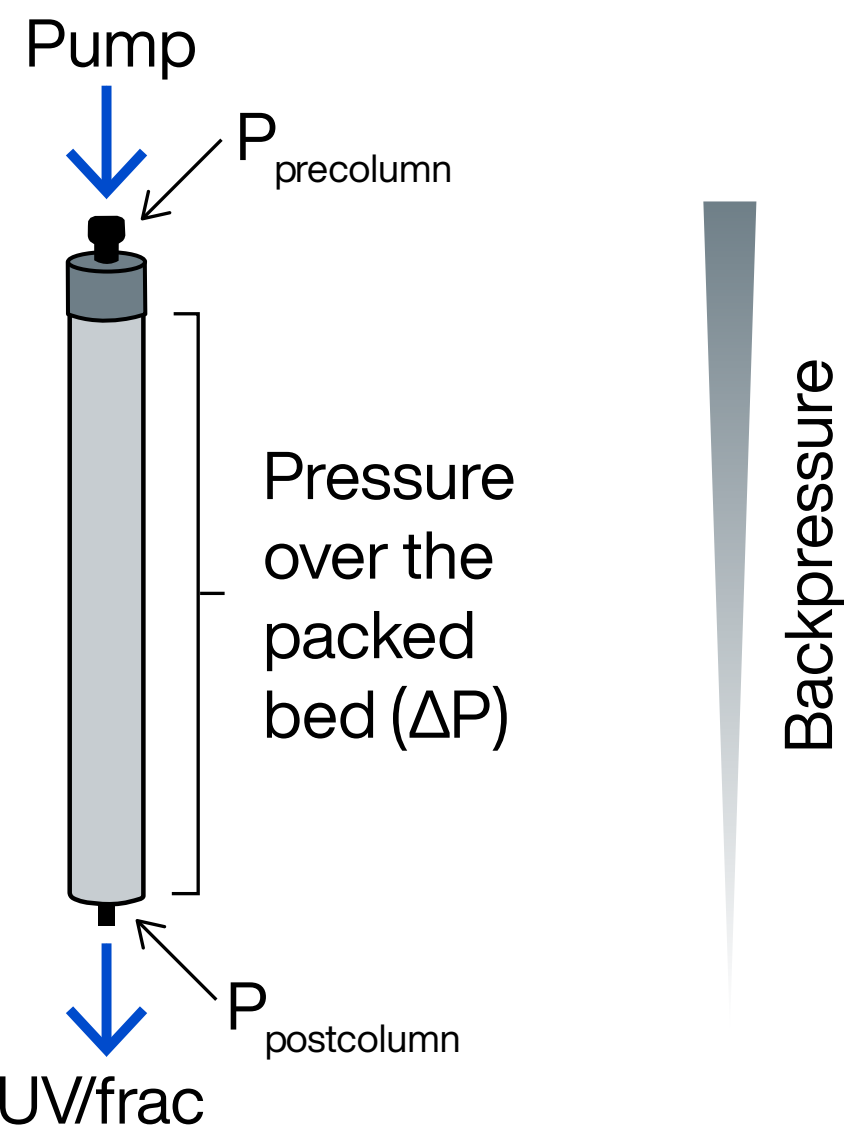


Figure 1. Illustration of total backpressure and individual pressures (P_{pre} , ΔP and P_{post}).

PoC study

The GoBio Mini Q 1 mL column (7x28 mm) was connected first to the LPLC system (Cytiva ÄKTA pure 25 with a restrictor connected) and then to the HPLC system (Shimadzu 2050-C) for testing on each platform.

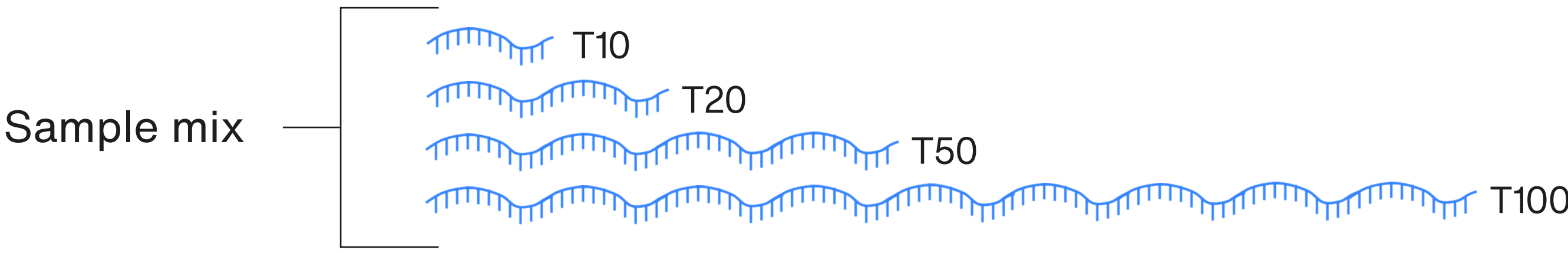
Oligonucleotide sequences of defined lengths were used as target molecules to be separated on the two systems using the same running conditions. The separations were evaluated based on the resolution between the peaks.

Resolution (R_s): The resolution considers factors, such as selectivity and efficiency (peak width) to determine the separation capacity between peaks. R_s should be as high as possible, and $R_s > 1.5$ usually means base line separation between peaks.

$$R_s = \frac{tR_{(2)} - tR_{(1)}}{0.5 (W_1 + W_2)}$$

Running conditions applied after optimizations

Column: GoBio Mini Q 1 mL (agarose-based AIEX resin)
Buffers: 20 mM tris-HCl, pH 8 (+ 1.5 M NaCl in elution buffer)
Flow rate: 0.5 mL/min (80cm/h)
Sample: Load of 25 µg DNA sequences in a mix per mL of resin
Elution: Linear gradient, 20% to 50% elution buffer in 20 column volumes (CV)

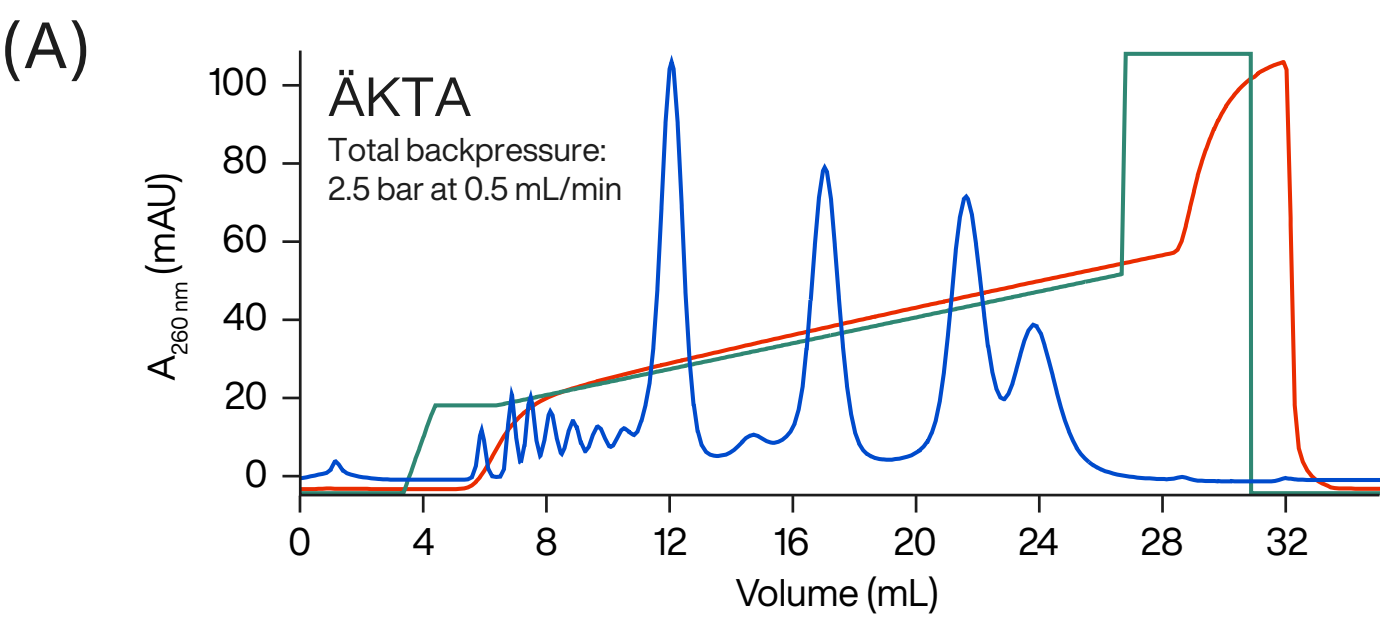


Results – HPLC compatibility of GoBio Mini 1 mL

- Separation:** The chromatograms generated on the ÄKTA and HPLC systems appeared nearly identical, showing similar peak resolutions for the DNA sequences.
- Backpressure:** Relatively similar between ÄKTA (with restrictor connected) and HPLC. All of the backpressure was contributed by the system.

To further validate the GoBio column compatibility with HPLC, two additional formats were tested: GoBio Mini 5 mL (13x38 mm, max pressure of 3 bar) and GoBio Screen 7x100 mm (3.8 mL, max pressure of 5 bar). Due to the larger column sizes, a higher sample load was applied, along with twice as high flow rate.

ÄKTA vs. HPLC: GoBio Mini 1 mL



HPLC: GoBio Mini (1 and 5 mL) vs. GoBio Screen

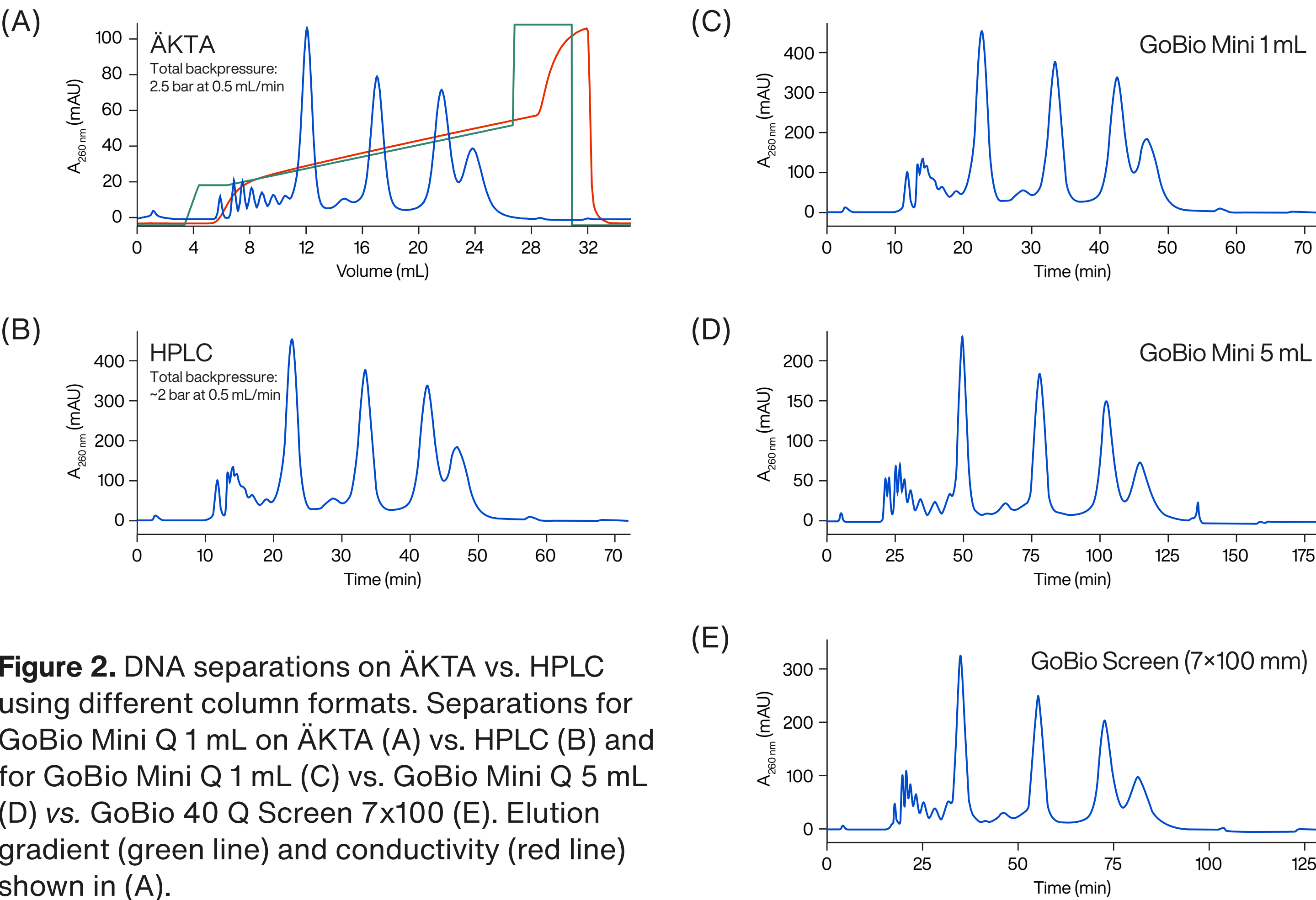


Figure 2. DNA separations on ÄKTA vs. HPLC using different column formats. Separations for GoBio Mini Q 1 mL on ÄKTA (A) vs. HPLC (B) and for GoBio Mini Q 1 mL (C) vs. GoBio Mini Q 5 mL (D) vs. GoBio 40 Q Screen 7x100 (E). Elution gradient (green line) and conductivity (red line) shown in (A).

Results – HPLC Compatibility of GoBio Mini 1 mL and 5 mL, and GoBio Screen

- Separation:** The resolution between peaks was similar to, or improved compared to, the separation on the ÄKTA system.
- Backpressure:** Most of the backpressure was contributed by the system. When the flow rate was doubled, the backpressure increased proportionally, indicating that very high flow rates may not be feasible.

Peaks	Resolution (R_s)			
	ÄKTA 1 mL	HPLC 1 mL	HPLC 5 mL	HPLC 3.8 mL
T10-T20	3.3	3.3	5.1	4.6
T20-T50	2.6	2.4	3.4	3.3
T50-T100	1.1	0.9	1.4	1.5

¹ R_s values were obtained by integrating peaks in Unicorn 7.1 or LabSolutions.

Conclusions of PoC study

Our results indicate that the selectivity and performance of agarose-based resins in HPLC are comparable to those obtained with traditional ÄKTA systems. This demonstrates the potential for purifying synthetic molecules, oligonucleotides, and peptides using more preparative IEX resins on an HPLC system.