HyPeak Chromatography System Gradient Performance

Introduction

This document details a study evaluating gradient performance of the Thermo Scientific™ HyPeak™ Chromatography System. Chromatography unit operations may incorporate discrete (step) and/or continuous (linear) gradients for a variety of process objectives. For instance, a linear gradient may aid in separating challenging impurities closely associated with the molecule of interest (e.g., aggregates for antibodies or empty capsids for viral vectors). Additionally, diluting buffer concentrates using a step gradient improves buffer volume management and reduces the required footprint for the unit operation. These design-focused solutions can function to build micro-efficiencies in the manufacturing workflow process, providing time and cost savings over the production life cycle.

The HyPeak Chromatography System offers a wide range of process scale flexibility using modular single-use fluid transfer assemblies available in four (4) inner diameters (ID) (1/4 in., 3/8 in.*, 1/2 in.*, and 3/4 in.) and up to three high-performance, low pulsation pumps (type and combination determined by processing needs). Binary and tertiary gradient capability is possible across the system flow rate ranges.

Materials and methods

The experiments were performed with the 3/4 in. ID fluid transfer assemblies as an example of the baseline expected gradient performance on the HyPeak Chromatography System. Table 1 shows the performance specifications for the sensors and pumps used during gradient testing. Simulated column backpressure of 2 bar was achieved by a manual restrictor. The bubble trap was inline to ensure appropriate mixing of the solution.

Table 1. Performance specifications for HyPeak sensors and pumps using 3/4 in. ID fluid transfer assemblies

Equipment	Specification
Quattroflow QF 1200SU 5°	20–1200 L/hr
Quattroflow QF 2500SU 5°	50–1980 L/hr
Levitronix LFS-15SU-SC1	0–3,000 L/hr
Optek Conductivity SUC-23	0–150 mS/cm
Optek UV 280 nm SUC-27	0–3 AU

*Fluid transfer assembly sizes are not immediately available. Please consult our technical support team for more information.

Step gradients

Step gradient tests were performed according to Table 2. BPCs containing concentrated sodium chloride (NaCl) and water were connected to the specified pumps. The system was operated at the target flow rate and % PU-10 output for 5 minutes.

Offline dilutions of the concentrated NaCl with water were tested using a benchtop meter. The inline conductivity results were compared to offline dilutions and the differences were reported.

Table 2. Step gradient test conditions using 3/4 in. fluid transfer assembly

Flow rate (L/hr)	Pump (type): solution	% Output PU-10
500	• PU-10 (QF 1200SU 5°): 2M NaCl • PU-20 (QF 1200SU 5°): Water	10 25 50
900	• PU-10 (QF 1200SU 5°): 2M NaCl • PU-20 (QF 1200SU 5°): Water	10 25 50
	• PU-10 (QF 1200SU 5°): 2M NaCl • PU-30 (QF 2500SU): Water	10 25 50
1,500	• PU-10 (QF 1200SU 5°): 2M NaCl • PU-30 (QF 2500SU): Water	10 25 50

Linear gradients

Linear gradients with acetone solution and concentrated NaCl were performed according to Tables 3 and 4, respectively. The linear gradient ranges at the various system flow rates considered the pump capabilities (Table 1). Pump flow outputs are based on programmed percentages of target flow rate. BPCs of acetone solution and water were connected to the specified pumps. As a practical example of linear gradients for ion-exchange operations, experiments were performed with concentrated NaCl while monitoring conductivity. Sodium chloride solution and water were connected to the specified pumps.

Gradient linear accuracy was assessed by comparing the HyPeak Chromatography System measurement to the programmed target.

Table 3. Acetone solution linear gradients test conditions using 3/4 in. fluid transfer assembly

Flow rate (L/hr)	Pump (type): solution	% Output PU-10
500	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water	10–90
900	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water	10–90
1,500	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water • PU-30 (QF 2500SU): Water	5–45*

*Same % output for PU-20

Table 4. Sodium chloride linear gradient test conditions using 3/4 in. fluid transfer assembly

Flow rate (L/hr)	Pump (type): solution	% Output PU-10
492	• PU-10 (QF 1200SU 5°): 0.5M NaCl • PU-20 (QF 1200SU 5°): Water	4–96
848	• PU-10 (QF 1200SU 5°): 0.5M NaCl • PU-20 (QF 1200SU 5°): Water	2–94

Results

Step gradients

Figures 1–4 show the inline conductivity measurements compared to offline dilutions of 2M NaCl with water at 10, 25, and 50%. The conductivity profile for the step gradients shows efficient mixing even at low flow rates without the need for an inline mixer.

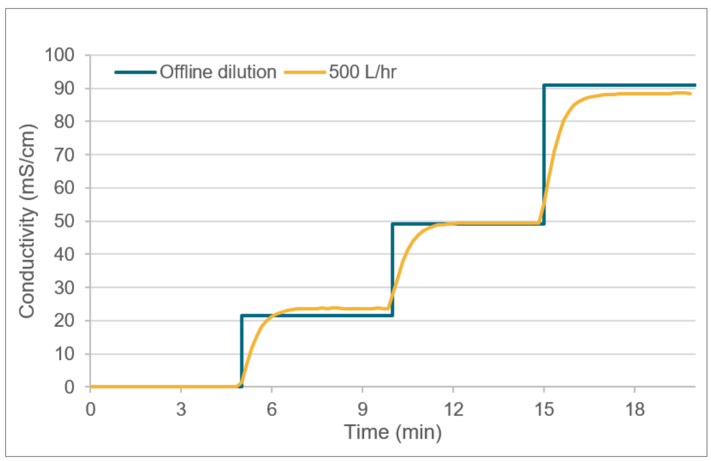

Figure 1. Step gradient result at a system flow rate of 500 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System conductivity (yellow) and offline dilutions (blue).

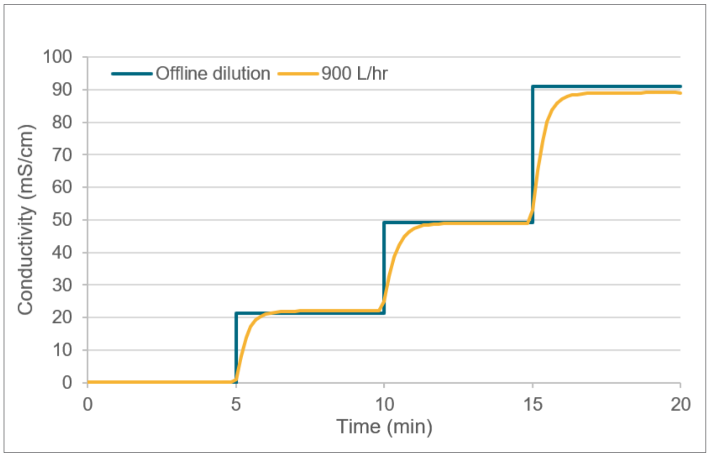

Figure 2. Step gradient result at a system flow rate of 900 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System conductivity (yellow) and offline dilutions (blue).

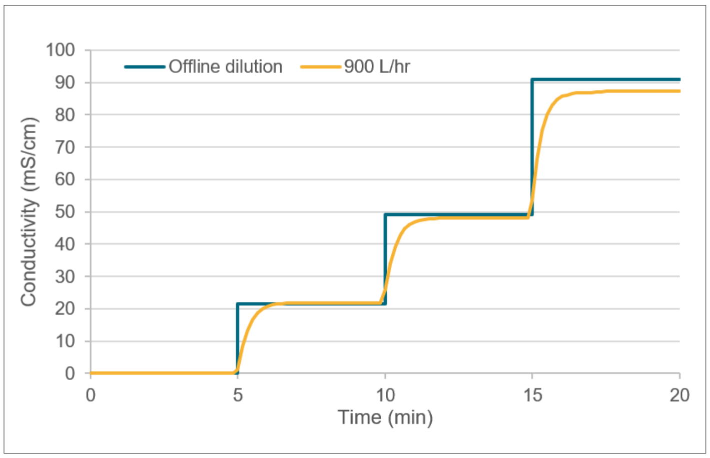

Figure 3. Step gradient result at a system flow rate of 900 L/hr using PU-10 (QF 1200SU) and PU-30 (QF 2500SU); HyPeak Chromatography System conductivity (yellow) and offline dilutions (blue).

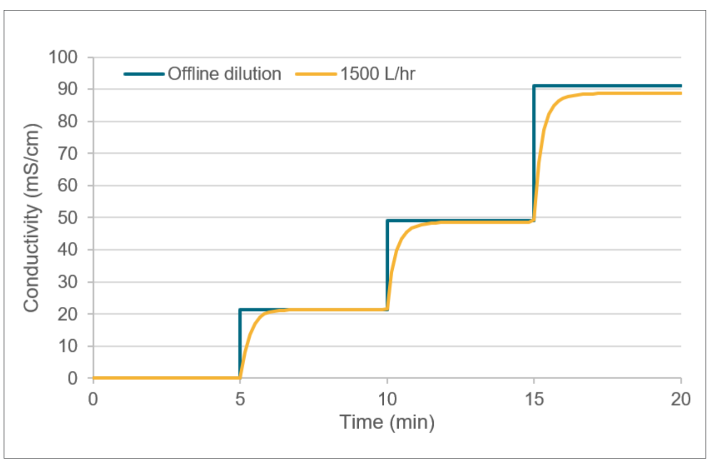

Figure 4. Step gradient result at a system flow rate of 1,500 L/hr using PU-10 (QF 1200SU) and PU-30 (QF 2500SU); HyPeak Chromatography System conductivity (yellow) and offline dilutions (blue).

Table 5 shows the percent difference between the HyPeak Chromatography System conductivity measurement and the offline dilution conductivity for the various test conditions. The HyPeak Chromatography System can provide efficient mixing and conductivity accuracy within 5% of target for most cases. While approximately 10% difference was observed at the 10% dilution at 500 L/hr, this operational scenario is considered unlikely with the 3/4 in. fluid transfer assembly as PU-10 is operating near the lower end of the flow rate range. For processes with lower system flow rates where a higher degree of accuracy is required, consider the smaller ID fluid transfer assemblies offered (1/4 in., 3/8 in., or 1/2 in., as appropriate) or using a higher primary component percentage to meet processing requirements. In the typical use case of inline dilution of buffer concentrates, ±10% of the target is the common range.

Table 5. Step gradient inline versus offline conductivity measurement difference

Pumps (type)	Flow rate (L/hr)	Conductivity % difference		
		10% PU-10 output	25% PU-10 output	50% PU-10 output
PU-10 and PU-20 (2 x QF 1200SU)	500	10.6	0.8	2.7
	900	3.1	0.7	2.1
PU-10 and PU-30 (2 x QF 1200SU and QF 2500SU)	900	1.6	2.1	3.9
	1,500	0.1	1.0	2.5

Linear gradients

Figures 5–7 show the inline UV absorbance measurements compared to percentage of PU-10 output. The UV absorbance profile at 280 nm for the linear gradients using acetone solution shows efficient mixing even at low flow rates without the need for an inline mixer. Linearity was exhibited at all three flow rates tested.

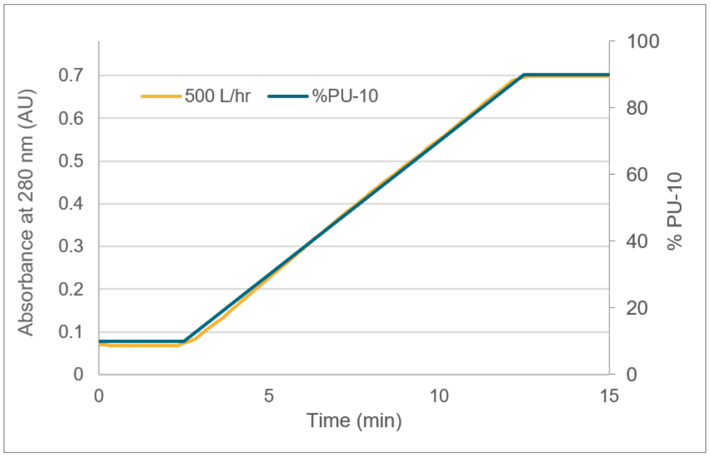

Figure 5. Linear gradient with acetone solution result at a system flow rate of 500 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System UV absorbance (yellow) and % PU-10 (blue).

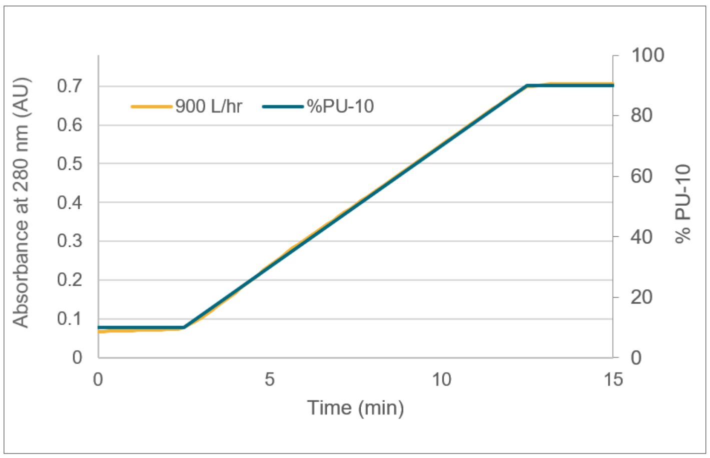

Figure 6. Linear gradient with acetone solution result at a system flow rate of 900 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System UV absorbance (yellow) and % PU-10 (blue).

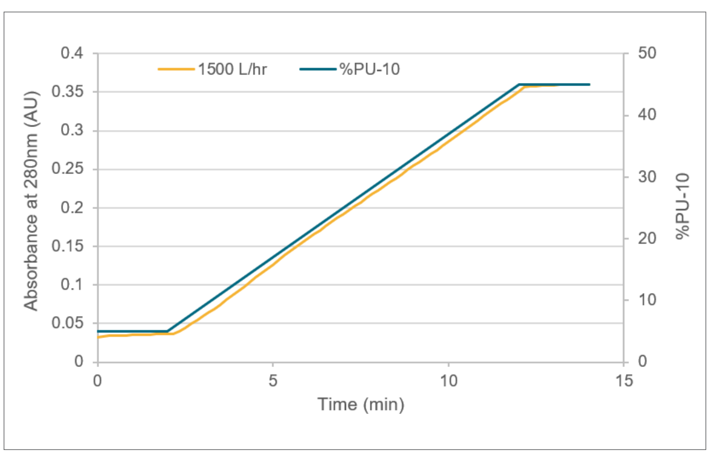

Figure 7. Linear gradient with acetone solution result at a system flow rate of 1,500 L/hr using PU-10, PU-20 (2 x QF 1200SU) and PU-30 (QF 2500SU); HyPeak Chromatography System UV absorbance (yellow) and % PU-10 (blue).

Table 6 shows the percent difference between the HyPeak Chromatography System UV measurement absorbance at 280 nm and the percentage of PU-10 output. The HyPeak Chromatography System can provide efficient mixing and linearity within 3% on average.

Table 6. Acetone linear gradient average percent linearity deviation

Flow rate (L/hr)	Pump (type): solution	% Linearity deviation average
480	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water	2%
900	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water	2%
1,500	• PU-10 (QF 1200SU 5°): 0.5M NaCl, 0.5% Acetone • PU-20 (QF 1200SU 5°): Water • PU-30 (QF 2500SU): Water	3%

Figures 8 and 9 show examples of conductivity gradients using 0.5M NaCl, which may be performed in an ion-exchange unit operation at 492 and 848 L/hr, respectively.

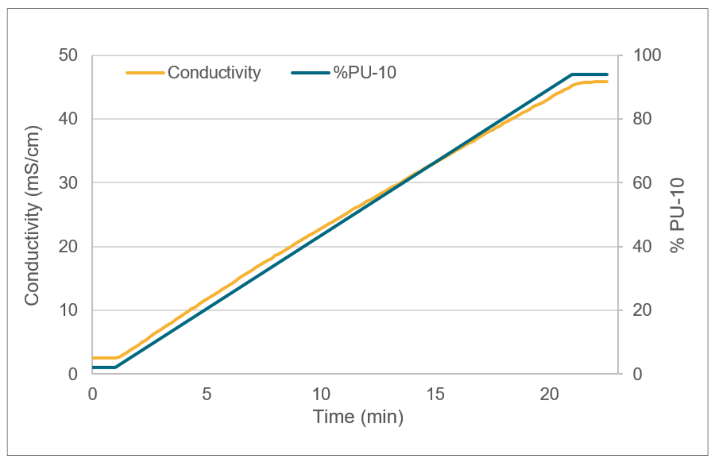

Figure 8. Linear gradient with 0.5M NaCl result at a system flow rate of 492 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System conductivity measurement (yellow) and % PU-10 (blue).

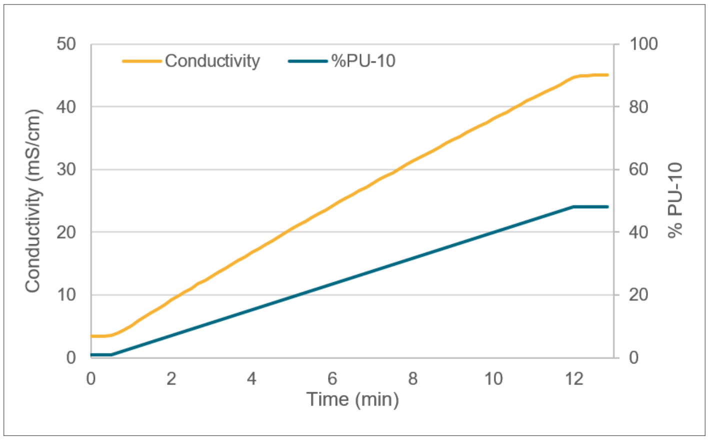

Figure 9. Linear gradient with 0.5M NaCl result at a system flow rate of 848 L/hr using PU-10 and PU-20 (2 x QF 1200SU); HyPeak Chromatography System conductivity measurement (yellow) and % PU-10 (blue).

Table 7 shows the average percent difference between the HyPeak Chromatography System conductivity measurement and offline dilutions. The HyPeak Chromatography System can provide efficient mixing and accuracy within 3% on average.

Table 7. Sodium chloride linear gradient average percent difference compared to offline dilution

Flow rate (L/hr)	Pump (type): solution	Average % difference (system vs offline dilution measurement)
492	• PU-10 (QF 1200SU 5°): 0.5M NaCl • PU-20 (QF 1200SU 5°): Water	3%
848	• PU-10 (QF 1200SU 5°): 0.5M NaCl • PU-20 (QF 1200SU 5°): Water	2%

Conclusion

These data demonstrate the step and linear gradient performance for the 3/4 in. ID fluid transfer assembly of the HyPeak Chromatography System. Gradient capability is a pivotal factor supporting operational efficiency and performance of downstream purification processes by enabling nuanced separations to meet product quality requirements or alleviating buffer footprint constraints. The step gradient accuracy was controllable at less than 5% error for realistic use cases with the 3/4 in. fluid transfer assembly. The acetone linear gradient average error was controllable at less than 3% on average for the working range of 10–90%. These results support the HyPeak Chromatography System's capability to produce accurate gradients.

TruChrom software design features include the ability to measure pH and conductivity pre- and post-column, allowing additional quality control oversight for the unit operation. Specifically, TruChrom allows users to set pH and conductivity limits for the pre-column sensors. When pre-column values are outside of user specified limits, the feed stream will bypass the column ensuring the column is only exposed to solutions with the intended pH and conductivity. This feature adds additional engineering controls when performing step gradients (i.e. inline dilution of buffer concentrates). Step and linear gradient data combined with design and software features offers operational efficiency with an enhanced user experience.

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