Separation Reproducibility With the BioLogic™ Chromatography Systems

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Introduction

In chromatographic separations, sample component elution times are often used both for identification and to program automated chromatography steps like fraction collection. Therefore, the ability to obtain reproducible chromatographic separations is essential. In order to achieve reproducible sample runs, a chromatography system must maintain a precise flow rate, inject a consistent sample volume at a precise time, and detect sample components in a consistent manner. Additionally, the column used must maintain its function and flow characteristics over time. In this report we provide data that shows the highly reproducible separations that can be obtained with both BioLogic DuoFlow™ and BioLogic LP™ systems. These results were obtained on a medium-pressure BioLogic DuoFlow QuadTec™ basic system and a low-pressure BioLogic LP™ system equipped with a BioFrac™ fraction collector.

Methods

A test mixture that contained the proteins horse skeletal muscle myoglobin, chicken egg white conalbumin, and soybean trypsin inhibitor type II (all obtained from Sigma) in a ratio of 2:5:5 was dissolved in an appropriate volume of 20 mM Tris buffer, pH 8.1 (buffer A), filtered through a 0.22 µm syringe filter, and kept on ice until injected onto a column.

The test mixture was purified in consecutive anion exchange runs using an UNO™ Q1 column or an Econo-Pac® High Q cartridge connected to the DuoFlow or BioLogic LP system, respectively. Proteins were eluted with a linear gradient of 0–50% buffer B (buffer A + 1 M NaCl). All buffers were filtered and degassed prior to use. For each run on the DuoFlow system, a 50 µl sample loop was overfilled with 150 µl of the protein sample using an Econo™ gradient pump. For the BioLogic LP system, 1 ml of sample was loaded though port C of the BioLogic LP buffer selection valve. The UV detector and conductivity monitor supplied with each system were used to monitor the UV signal and conductivity, respectively. Protocol details are summarized in Tables 1 and 2 and the system components used are listed in Table 3.

Table 1. Separation protocol on the BioLogic DuoFlow system.

	Description	Buffer Composition	Volume, Flow Rate
	Anion exchange chromatography		
1.	Isocratic flow	Buffer A 100%	0.4 ml at 2.0 ml/min
2.	Zero baseline for QuadTec		
3.	Isocratic flow	Buffer A 100%	1.0 ml at 2.0 ml/min
4.	Load/inject with auxiliary pump fill		0.2 ml at 1.0 ml/min
5.	Isocratic flow	Buffer A 100%	2.0 ml at 2.0 ml/min
6.	Linear gradient	Buffer A 100 to 50% Buffer B 0 to 50%	20.0 ml at 2.0 ml/min
7.	Isocratic flow	Buffer A 50% Buffer B 50%	5.0 ml at 2.0 ml/min
	Column equilibration		
1.	Isocratic flow	Buffer B 100%	4.0 ml at 2.0 ml/min
2.	Isocratic flow	Buffer A 100%	2.0 ml at 2.0 ml/min
3.	Hold until conductivity <2.7 mS/cm	Buffer A 100%	2.0 ml/min
4.	Hold until <0.1 AU at 280 nm	Buffer A 100%	2.0 ml/min
5.	Isocratic flow	Buffer A 100%	4.0 ml at 2.0 ml/min
6.	Zero baseline for QuadTec		
7.	Isocratic flow	Buffer A 100%	1.0 ml at 2.0 ml/min

Table 2. Anion exchange chromatography protocol on the BioLogic LP system.

	Buffer Composition	Volume, Flow Rate
1.	Buffer A	1 ml at 1.5 ml/min
2.	Sample	1 ml at 1.5 ml/min
3.	Buffer A	5 ml at 1.5 ml/min
4.	Linear gradient of 0–50% buffer B	20.0 ml at 1.5 ml/min
5.	Buffer B	5.0 ml at 1.5 ml/min
6.	Buffer A	15 ml at 1.5 ml/min

Table 3. Comparison of system components used to test reproducibility.

	BioLogic DuoFlow	BioLogic LP
Column	UNO Q1 column	Econo-Pac High Q cartridge
Protein load	300 µg/50 µl	900 µg/1 ml
Loading method	Sample loop	Buffer selection valve
UV detector	BioLogic QuadTec	BioLogic LP optics module

Results

The BioLogic DuoFlow and BioLogic LP systems were used to successfully purify a protein test mixture with excellent reproducibility. A total of 80 ion exchange runs were performed on the BioLogic DuoFlow QuadTec system as a queue of alternating anion exchange and equilibration runs (160 runs total). As shown in Figure 1, the 80 separations were virtually indistinguishable. The observed protein retention times (Table 4) demonstrate consistent sample introduction and pump performance. The standard deviation associated with the gradient slope, 4.88 ± 0.01 mS/cm/min, indicates excellent pump and mixer performance, which is critical for discrete protein resolution. These results confirm that the BioLogic DuoFlow chromatography system provides high run-to-run reproducibility.

Table 4. Retention times for test proteins separated on Bio-Rad chromatography systems. Shown are average retention times ± SD for 80 consecutive runs for the BioLogic DuoFlow and 25 for the BioLogic LP system.

	BioLogic DuoFlow	BioLogic LP
Myoglobin	1.48 ± 0.02 min	3.67 ± 0.02 min
Conalbumin	4.54 ± 0.01 min	10.92 ± 0.04 min
Soybean trypsin inhibitor	7.38 ± 0.01 min	15.10 ± 0.05 min

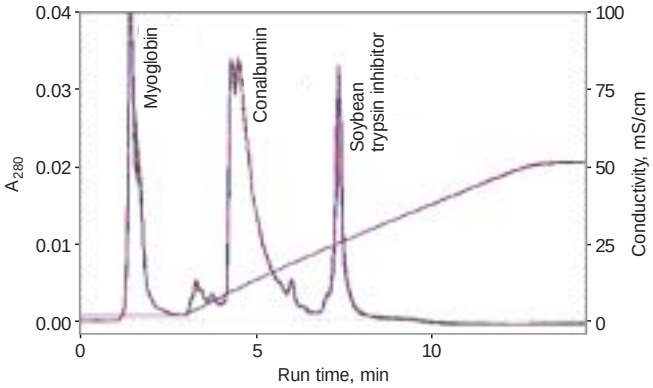

Fig. 1. Reproducibility of a BioLogic DuoFlow QuadTec system. Shown is an overlay of 80 runs. Red line, conductivity trace.

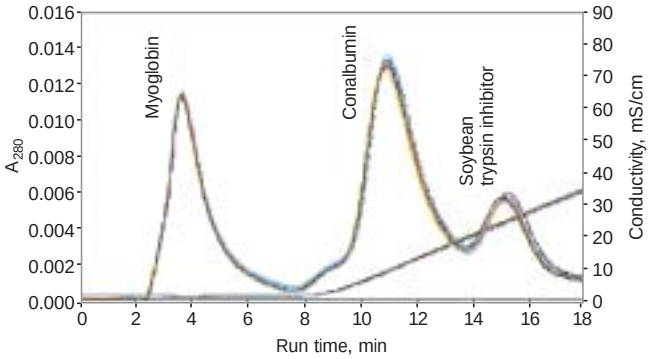

Fig. 2. Reproducibility of a BioLogic LP system. Shown is an overlay of 25 consecutive separations using the multi-run feature.

On the BioLogic LP, 25 consecutive separations were performed using its Multirun feature. As shown in Figure 2, the separations were highly reproducible and exhibited extremely consistent protein elution times (Table 4). Gradient performance was also highly reproducible with a small standard deviation (Figure 2); the observed average gradient slope was 3.59 ± 0.05 mS/cm/min. These results show that the BioLogic LP gives the consistent mixing and reliable pump performance required from a low-pressure chromatography system.

Conclusions

These results demonstrate that the BioLogic DuoFlow and BioLogic LP chromatography systems routinely deliver highly reproducible results. This high level of reproducibility is critical for obtaining consistent sample purity and for automating laboratory chromatography processes. Whether your separation requirements are analytical or preparative, the BioLogic DuoFlow and BioLogic LP chromatography systems provide high-performance, reliable results.

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