YMC EcoPrime® LPLC with Enhanced Buffer In-line Dilution

Chromatography and buffer in-line dilution are integrated on the same system combining two unit operations into a single, space-saving platform. The EcoPrime buffer in-line dilution (BID) option will significantly reduce buffer storage and lower operating expenses associated with buffer preparation.

Introduction

Enhanced Buffer In-line Dilution (BID) is the use of three pumps to perform buffer in-line dilution while executing a chromatography process step in either isocratic step or linear gradient mode. When performing BID, buffer(s) are supplied to the system at a concentration greater than the required buffer concentration for the process step and diluted. For example, the concentrated buffers could be supplied at a concentration of five (5) times the required concentration (5x).

While the two (2) concentrated buffers are being supplied, the third pump delivers a diluent solution (typically purified water or WFI), diluting the concentrated buffers to the concentration required for the chromatography process step (Figure 1).

This provides the user with the added functionality of executing chromatographic processes with concentrated buffers, saving the labor associated with buffer preparation and the space typically used for storing ready-to-use buffers.

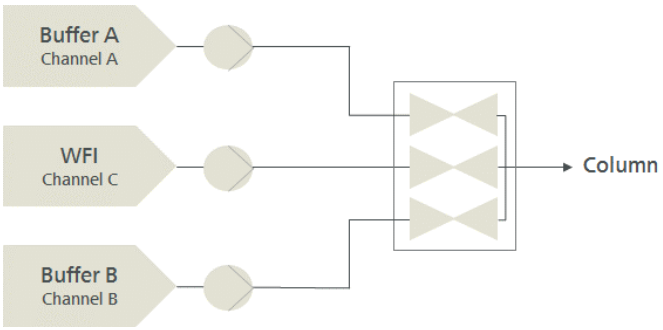

Figure 1. Basic Flow Diagram for Buffer In-line Dilution

This white paper describes how the EcoPrime LPLC with the enhanced BID option can be used to perform buffer in-line dilution prior to chromatography. Several examples with isocratic and gradient elution and BID are presented.

Buffer Considerations

Care must be taken to ensure that the buffer concentrates are no so concentrated that they precipitate out of solution in the system prior to be diluted. It is good practice to determine the solubility of each buffer prior to use on the system.

The effect of the buffer concentration on the materials-of-construction should also be considered. For example, high sodium chloride (NaCl) concentrations may cause pitting and corrosion to the 316L SS product contact components if the contact time is unusually long or the solution remains stagnate in the system. It is good practice to adequately flush the system with water after using potentially damaging buffer solutions.

An advantage of using enhanced BID is that buffer concentrates in bags can be used to supply the system. If single-use bags are used, the buffer bag tubing must be sized appropriately. Even at very low flow rates, tubing connections that are too small can result in pump performance issues such as pump cavitation.

Configuring BID with EcoPrime Software

EcoPrime LPLC software has two different methods for programming a dilution; **Fixed Percentage** or **Buffer Concentration Factor**.

> **Fixed Percentage**: allows the user to specify the percentage of the total flow rate that is to be supplied by the dilution pump.
>
> **Buffer Concentration Factor**: allows the user to specify the supply and target concentration factors of the concentrate streams.

When using the **Buffer Concentration Factor** methodology, both concentrates must have the same supply and target

concentration factors. The software does not allow the user to specify individual concentrates and target concentration factors. A single supply and target concentration factor is used for both concentrate streams.

Both **Fixed Percentage** and **Buffer Concentration Factor** allow the same concentrate to be used in different process steps at different concentrations.

Isocratic Step Elution with Enhanced BID

Buffer In-line Dilution is used to dilute two buffers (1:10 dilution) for an isocratic step elution. The total flow rate is 5.0 LPM.

Pump C, the diluent pump, will deliver 90% of the total flow (4.5 LPM) to dilute the two buffers (Buffer A and Buffer B). When configuring the percentages of concentrate buffers, together they must equal the balance of the total flow (10% or 0.5 LPM). See the table (below) with flow rates and buffer and diluent percentages.

Software configuration

To define the concentrate Isocratic Step percentages, the isocratic step concentrate buffer #1 and #2 percentages are always defined in terms of the percentage of the total flow rate (Figure 2).

Table. 1:10 Dilution, Isocratic Step Elution

Step #	Channel C (diluent)	Channel A (Buffer A)	Channel B (Buffer B)	Total Flow % and Rate	Channel A Ratio to Channel A/B Flow	Channel B Ratio to Channel A/B Flow
1	90% 4.5 LPM	10% 0.5 LPM	0% 0 LPM	100% 5.0 LPM	100%	0%
2	90% 4.5 LPM	8.5% 0.425 LPM	1.5% 0.075 LPM	100% 5.0 LPM	85%	15%
3	90% 4.5 LPM	5% 0.25 LPM	5% 0.25 LPM	100% 5.0 LPM	50%	50%
4	90% 4.5 LPM	1.5% 0.075 LPM	8.5% 0.425 LPM	100% 5.0 LPM	15%	85%
5	90% 4.5 LPM	0% 0 LPM	10% 0.5 LPM	100% 5.0 LPM	0%	100%

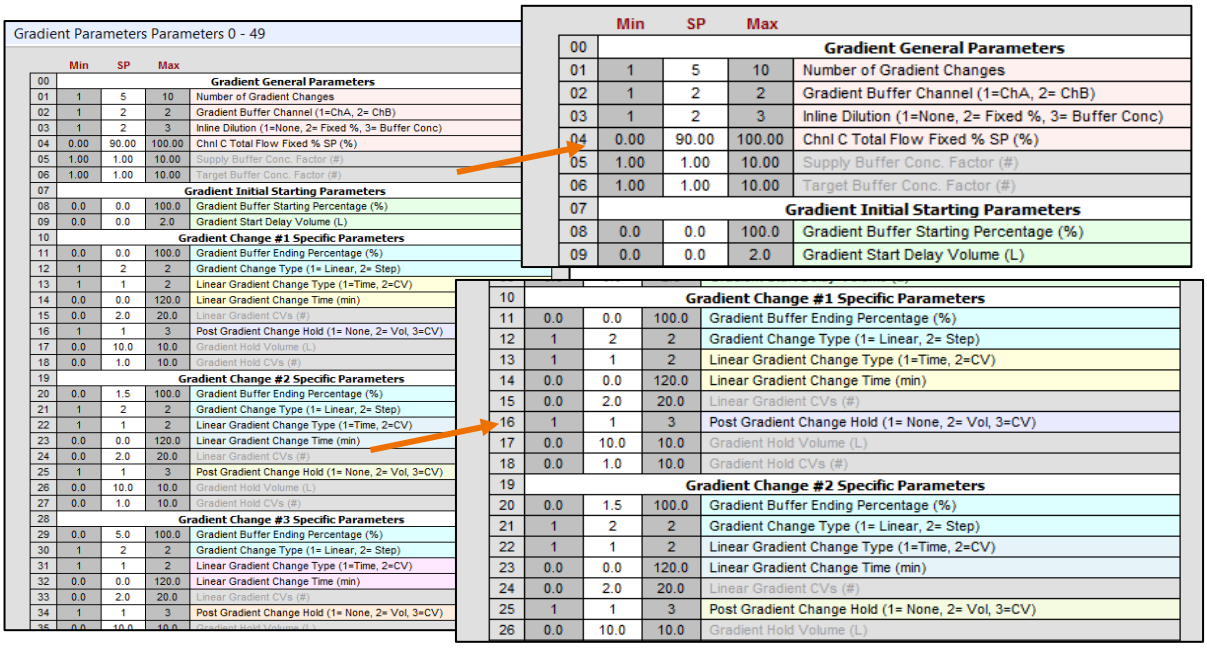

Figure 2. Isocratic Step Parameters screen showing Concentrate Buffer Percentages

Gradient Elution with Enhanced BID

In this example, Buffer In-line Dilution is used to dilute two buffers (1:10 dilution) for a 3 step gradient elution that includes one linear segment followed by two isocratic steps. The total flow rate is 5.0 LPM.

Pump C, the diluent pump, will deliver 90% of the total flow (4.5 LPM) to dilute the two buffers (Buffer A and Buffer B).

Software configuration

When configuring the percentages of concentrate buffers, the gradient (linear and step) starting and ending percentages **<u>are</u>** **<u>always</u>** defined in terms of the percentage of the **<u>gradient</u>** flow rate of the system.

Buffer B will be the specified **Gradient Buffer** and the EcoPrime software calculates the percentage of Buffer A.

Table. 1:10 Dilution, Linear and Step Gradient

Gradient Change #	Channel C (diluent)	Channel B (specified)		Channel A (calculated)		Channel A, B Totals
		Starting	Ending	Starting	Ending	
1	90%	0%	50%	100%	50%	10% of total flow 0.50 LPM
	4.50 LPM	0.00 LPM	0.25 LPM	0.50 LPM	0.25 LPM	
		Linear Gradient				
2	90%	50%	75%	50%	25%	10% of total flow 0.50 LPM
	4.50 LPM	0.25 LPM	0.375 LPM	0.25 LPM	0.125 LPM	
		Step Gradient				
3	90%	75%	100%	25%	0%	10% of total flow 0.50 LPM
	4.50 LPM	0.375 LPM	0.50 LPM	0.125 LPM	0.00 LPM	
		Step Gradient				

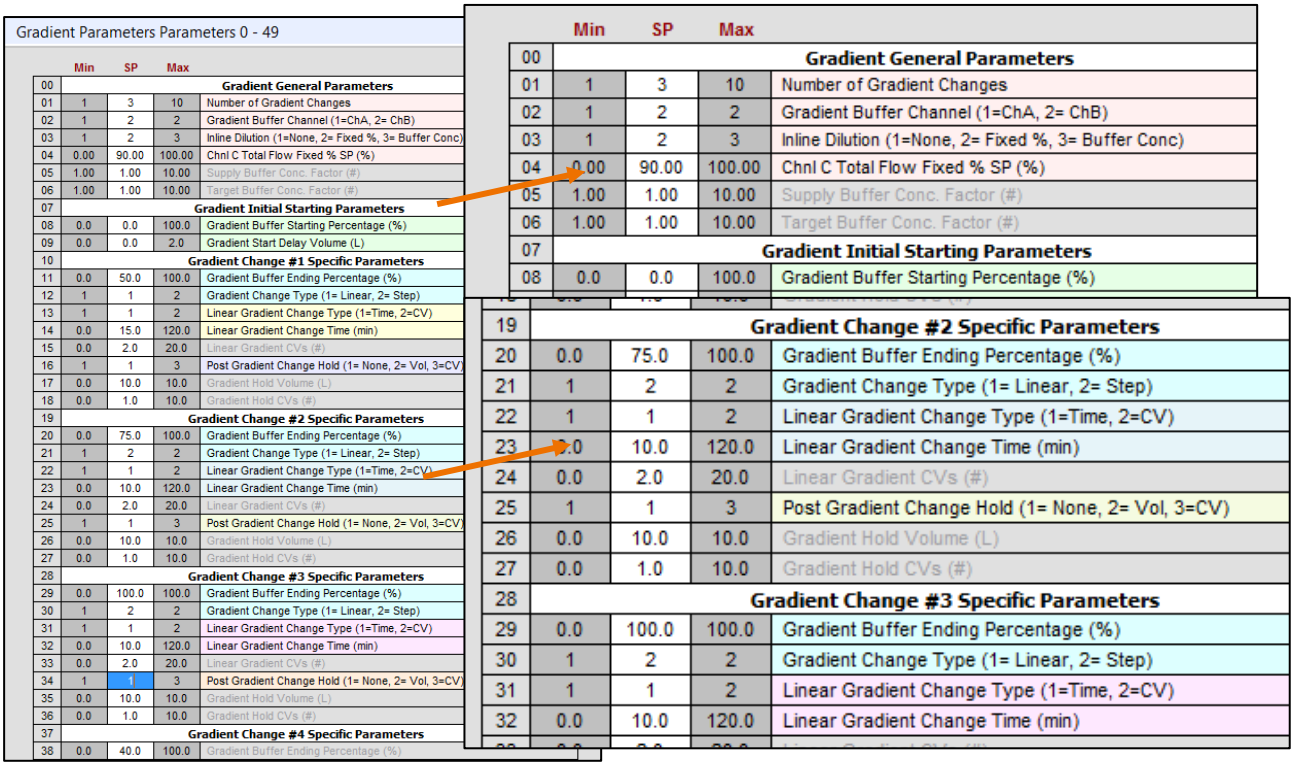

Figure 3. Gradient Parameters screen showing Fixed % Dilution Method with 90% Channel C (Diluent) and Gradient Buffer (Buffer B) Percentages in 3-step gradient elution

Exceptional Flow Accuracy and Precision

The EcoPrime LPLC system with enhanced BID utilizes YMC's digital fluid control with state-of-the-art pumps and exclusive digital pump control technology providing the highest volumetric flow precision and accuracy available today. The precise and accurate flow of the ecodos® metering pumps used in the system enables dilution factors up to 150 to 1 with concentration errors of less than 0.25% (Figure 4).

As a result of the exceptional flow rate performance, there is no need to rely on in-line pH or conductivity probes that have an inherent tendency to drift and require frequent calibration. With the YMC approach, using the volumetric

flow performance of our metering pumps to blend buffer concentrate and diluent, you eliminate process variables assuring better control and resulting in a more simple system that can be easily validated.

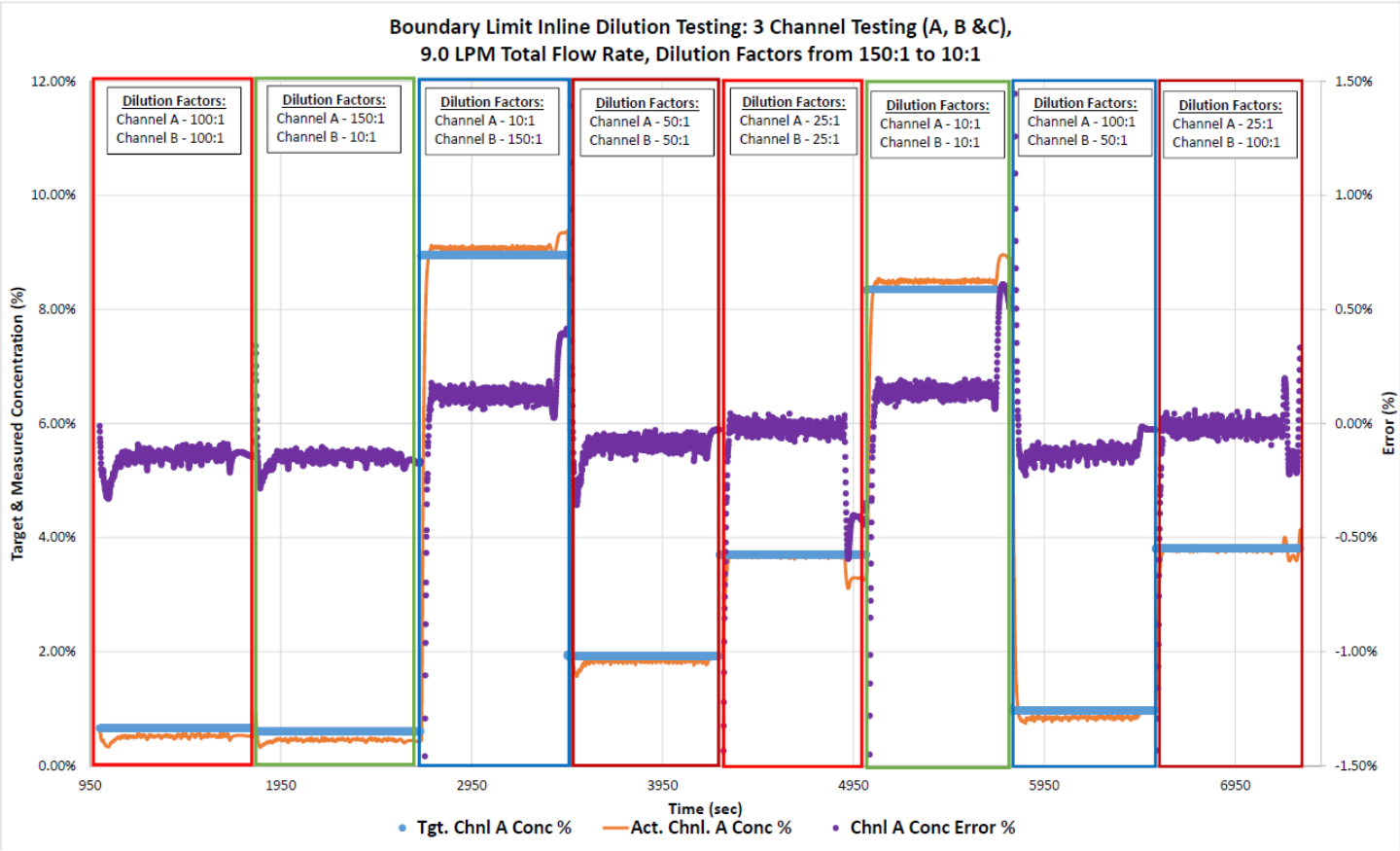

Figure 4. EcoPrime LPLC Enhanced BID Dilution Factors (10x, 25x, 50x, 100x, and 150) and associated Error % (concentration).

Ordering information

To order the EcoPrime BID system, please contact your regional sales representative.

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