

YMC EcoPrime® LPLC with Enhanced Buffer In-line Dilution

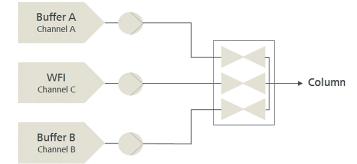
Chromatography and buffer in-line dilution are integrated on the same system combining two unit operations into a single, space-saving platform. The EcoPrime buffer in-line dilution (BID) option will significantly reduce buffer storage and lower operating expenses associated with buffer preparation.

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. Dilution factors up to 150 to 1 can be achieved with the EcoPrime LPLC with enhanced BID.

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.





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 not so concentrated that they precipitate out of solution in the system prior to be diluted. Therefore, 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 aggressive 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 that can severely affect the separation.

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** method and diluting two concentrates, 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 at different concentrations in different steps in a recipe.

Example: Isocratic Step Elution with Enhanced BID

In this example, 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 <u>are always</u> 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%

00	Min		raran						Min	SF		Max	
01	Min		adient Parameters Parameters 0 - 49					00					Gradient General Parameters
01							01	1	5		10	Number of Gradient Changes	
	00 Gradient General Parameters						02	1	2	-		Gradient Buffer Channel (1=ChA, 2= ChB)	
		1 5 10 Number of Gradient Changes				_	02	1	_	-	2		
02	1	2	2	Gradient Buffer Channel (1=ChA, 2= ChB)				03	1	2	- 1	3	Inline Dilution (1=None, 2= Fixed %, 3= Buffer Conc
03	1	2	3	Inline Dilution (1=None, 2= Fixed %, 3= Buffer Conc)				04	0.00	90.0	10	100.00	ChnI C Total Flow Fixed % SP (%)
	0.00	90.00	100.00	Chril C Total Flow Fixed % SP (%) Supply Buffer Conc. Factor (#)			-	\rightarrow		_	-		
	1.00	1.00	10.00	Supply Buffer Conc. Factor (#) Target Buffer Conc. Factor (#)	-		1	05	1.00	1.0	0	10.00	Supply Buffer Conc. Factor (#)
06	1,00	1,00		Gradient Initial Starting Parameters	-	-	(06	1.00	1.0	0	10.00	Target Buffer Conc. Factor (#)
08	0.0	0.0	100.0			-	E	07					
08 0.0 0.0 100.0 Gradient Buffer Starting Percentage (%) 09 0.0 0.0 2.0 Gradient Start Delay Volume (L)							_	Gradient Initial Starting Parameters					
10	0.0	0.0		radient Change #1 Specific Parameters				08	0.0	0.0	0	100.0	Gradient Buffer Starting Percentage (%)
11	0.0	0.0	100.0	Gradient Buffer Ending Percentage (%)				09	0.0	0.0		2.0	Gradient Start Delay Volume (L)
12	1	2	2	Gradient Change Type (1- Linear, 2- Step)		_	_		0.0				
13	1	1	2	Linear Gradient Change Type (1=Time, 2=CV)		-					_		,
14	0.0	0.0	120.0	Linear Gradient Change Time (min) 10						Gi	radient Change #1 Specific Parameters		
15	0.0	2.0	20.0	Linear Gradient CVs (#)	1 [11	0.0		0.0	100.0	Grad	dient Buff	er Ending Percentage (%)
16	1	1	3	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)		12	1		2	2	Grad	diant Char	nge Type (1= Linear, 2= Step)
17	0.0	10.0	10.0	Gradient Hold Volume (L)		_	<u>.</u>		1				
18	0.0	1.0					1			2			nt Change Type (1=Time, 2=CV)
	19 Gradient Change #2 Specific Parameters 14				14	0.0		0.0	120.0	Linear Gradient Change Time (min)			
20	0.0	1.5	100.0	Gradient Buffer Ending Percentage (%)	- F	15	0.0		2.0	20.0	Linear Gradient CVs (#)		
21 22	1	2	2	Gradient Change Type (1= Linear, 2= Step)		16	1		1	3	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)		
22	0.0	0.0	120.0	Linear Gradient Change Type (1-Time, 2-CV) Linear Gradient Change Time (min)		17	0.0		10.0	10.0			
24	0.0	2.0	20.0	Linear Gradient CVs (#)	1 -			_					
25	1	1	3	Post Gradient Change Hold (1= None, 2= Vol. 3+CV)	1 1	18	0.0		1.0	10.0	Grad	dient Hold	CVs (#)
28	0.0	10.0	10.0	Gradient Hold Volume (L)		19		Gradient Change #2 Specific Parameters					
27	0.0	1.0	10.0	Gradient Hold CVs (#)	1 1	20	0.0		1.5	100.0	Grad	dient Buff	er Ending Percentage (%)
28 Gradient Change #3 Specific Parameters			21	4		2	2	_		nge Type (1= Linear, 2= Step)			
29	0.0	5.0	100.0	Gradient Buffer Ending Percentage (%)	1 -	_	-		_				
30	1	2	2	Gradient Change Type (1= Linear, 2= Step)		22	1		1	2	_		nt Change Type (1=Time, 2=CV)
31	1	1	2	Linear Gradient Change Type (1=Time, 2=CV)		23	0.0		0.0	120.0	Linear Gradient Change Time (min)		nt Change Time (min)
32	0.0	0.0	120.0	Linear Gradient Change Time (min)	1	24	0.0		2.0	20.0	Linear Gradient CVs (#)		nt CVs (#)
33	0.0	2.0	20.0	Linear Gradient CVs (#)	1 1	25	1		1	3	_		Change Hold (1= None, 2= Vol, 3=CV)
34	1	1	3	Post Gradient Change Hold (1= None, 2= Vol, 3+CV)		25	0.0		10.0	10.0	Gradient Hold Volume (L)		

Figure 2. Isocratic Step Parameters screen showing Concentrate Buffer Percentages

Example: 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 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.

Gradient	Channel C	Channel B (specified)	Channel A (c	alculated)	Channel A, B Totals	
Change #	(diluent)	Starting	Ending	Starting	Ending		
1	90%	0%	50%	100%	50%	10% of total flow	
T	4.50 LPM	0.00 LPM	0.25 LPM	0.50 LPM	0.25 LPM	0.50 LPM	
			0.50 LPM				
2	90%	50%	75%	50%	25%	10% of total flow	
2	4.50 LPM	0.25 LPM	0.375 LPM	0.25 LPM	0.125 LPM	0.50 LPM	
			Step	Gradient		0.50 LFM	
	90%	0% 75%		25%	0%	10% of total flow	
3	4.50 LPM	0.375 LPM	0.375 LPM 0.50 LPM		0.00 LPM	0.50 LPM	
			Step		0.30 LFM		

Table. 1:10 Dilution, Linear and Step Gradient

adier	nt Para	meters	Param	neters 0 - 49			Min	SP	Max			
Min SP Max					00	Gradient General Parameters						
00				Gradient General Parameters		01	1	3	10	Number of Gradient Changes		
01	1	3	10	Number of Gradient Changes		02 1		2	2	Gradient Buffer Channel (1=ChA, 2= ChB)		
02	1	2	2	Gradient Buffer Channel (1=ChA, 2= ChB)								
03	1	2	3	Inline Dilution (1=None, 2= Fixed %, 3= Buffer Conc)		03 1		2	3	Inline Dilution (1=None, 2= Fixed %, 3= Buffer Conc)		
04	0.00	90.00	100.00	Chnl C Total Flow Fixed % SP (%)		04 0.00		90.00	100.00	Chnl C Total Flow Fixed % SP (%)		
05	1.00	1.00	10.00	Supply Buffer Conc. Factor (#)			_					
06	1.00	1.00	10.00	Target Buffer Conc. Factor (#)		05	1.00	1.00	10.00	Supply Buffer Conc. Factor (#)		
07				06	1.00	1.00	10.00	Target Buffer Conc. Factor (#)				
08	0.0	0.0	100.0	Gradient Buffer Starting Percentage (%)			1.00	1.00	10.00	Talger Duffer Collo, Lactor (#)		
10	09 0.0 0.0 2.0 Gradient Start Delay Volume (L)					07	07 Gradient Initial Starting Parameters					
_	0.0	50.0		adient Change #1 Specific Parameters		08 0.0		0.0	100.0 Gradient Buffer Starting Percentage (%)			
11 12	0.0	50.0	100.0	Gradient Buffer Ending Percentage (%)		00	0.0	0.0	100.0	Gradient Burrer Starting Percentage (%)		
13	1		2	Gradient Change Type (1= Linear, 2= Step) Linear Gradient Change Type (1=Time, 2=CV)			0.0	1.0	10.0			
14	0.0	15.0	120.0	Linear Gradient Change Type (1=1ine, 2=CV)	19 Gradient Change #2 Specific Parameters					radient Change #2 Specific Parameters		
14	0.0	2.0	20.0	Linear Gradient CVs (#)								
16	1	2.0	3	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)	20	0	0.0	75.0	100.0	Gradient Buffer Ending Percentage (%)		
17	0.0	10.0	10.0	Gradient Hold Volume (L)	2	1	1	2	2	Gradient Change Type (1= Linear, 2= Step)		
18	0.0	1.0	10.0	Gradient Hold CVs (#)	4		1	2	2	Gradient Change Type (1= Linear, 2= Step)		
19			2	2	1	1	2	Linear Gradient Change Type (1=Time, 2=CV)				
20	0.0	75.0	100.0	Gradient Buffer Ending Percentage (%)								
21	1	2	2	Gradient Change Type (1= Linear, 2= Step)	23	3	0.0	10.0	120.0	Linear Gradient Change Time (min)		
22	1	1	2	Linear Gradient Change Type (1=Time, 2=CV)	24	4	0.0	2.0	20.0	Linear Gradient CVs (#)		
23	0.0	10.0	120.0	Linear Gradient Change Time (min)		_	0.0	2.0		Linear Gradieni CVS (#)		
24	0.0	2.0	20.0	Linear Gradient CVs (#)	25	5	1	1	3	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)		
25	1	1	3	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)								
26	0.0	10.0	10.0	Gradient Hold Volume (L)	20	5	0.0	10.0	10.0	Gradient Hold Volume (L)		
27	0.0	1.0	10.0	Gradient Hold CVs (#)	2	7	0.0	1.0	10.0	Gradient Hold CVs (#)		
-	28 Gradient Change #3 Specific Parameters				21	r	0.0 1.0 10.0 Gradient Hold CVS (#)					
29	0.0	100.0	100.0	Gradient Buffer Ending Percentage (%)	28				Gradient Change #3 Specific Parameters			
30	1	2	2	Gradient Change Type (1= Linear, 2= Step)				100.0				
31	1	1	2	Linear Gradient Change Type (1=Time, 2=CV)	29	9	0.0	100.0	100.0	Gradient Buffer Ending Percentage (%)		
32	0.0	10.0	120.0	Linear Gradient Change Time (min)	30	n	1	2	2	Gradient Change Type (1= Linear, 2= Step)		
33	0.0	2.0	20.0	Linear Gradient CVs (#)				~				
34	1	1	-	Post Gradient Change Hold (1= None, 2= Vol, 3=CV)	3	31 1		1	2	Linear Gradient Change Type (1=Time, 2=CV)		
35 36	0.0	10.0	10.0	Gradient Hold Volume (L)	_							
36	0.0	1.0	10.0	Gradient Hold CVs (#)	32	2	0.0	10.0	120.0	Linear Gradient Change Time (min)		
37	0.0	40.0	Gr 100.0	adient Change #4 Specific Parameters Gradient Buffer Ending Percentage (%)	-		0.0	0.0	00.0			

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 digital fluid control with state-of-the-art pumps and exclusive digital pump control technology (LEWA GmbH) 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 volumetric flow error of less than 0.5% (Figure 4).

Using accurate and precise volumetric flow to prepare point-of-use buffers from buffer concentrates ensures predictable ion concentrations (buffer composition) and pH control.

Conclusion

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. Using the volumetric flow performance of the 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.

- Assure better control of buffer composition
- Minimize process variables (ion concentration, pH, and conductivity)
- Facilitate validation with straight-forward process controls

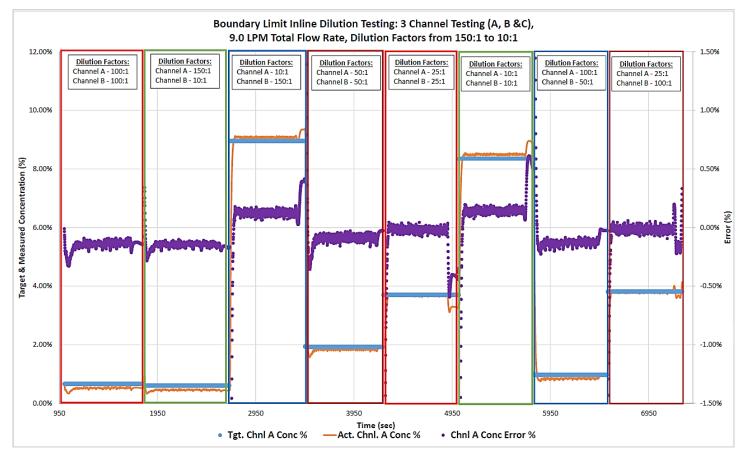
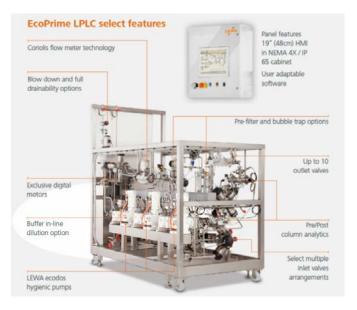
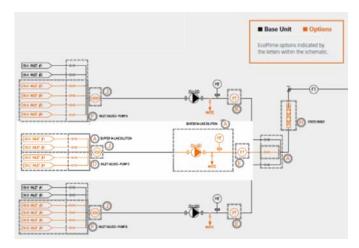


Figure 4. EcoPrime LPLC with Enhanced BID achieves volumetric flow accuracy better than 1.0 % for Dilution Factors of 10x, 25x, 50x, 100x, and 150x

- Channels A and B are the buffer concentrate channels. Channel C is the diluent channel.
- Data shown are for Channel A. Channel B data are not shown.
- Flow rate percentages are determined by measuring tryptophan UV signal at 280nm. The total system flow rate is 9.00 LPM.
- "Target & Measured Concentration (%)" (x-axis, left) are the Target and Actual Channel A Buffer Concentrate flow rate percentages.
- "Error" (x-axis, right) is Target Actual Channel A Buffer Concentrate flow rate percentages.
- "Tgt. Chnl. A Conc %" is the Target Channel A Buffer Concentrate flow rate percentage.
- "Act. Chnl. A Conc %" is the Actual Channel A Buffer Concentrate flow rate percentage.
- "Chnl A Conc Error %" is the Target Actual Channel A Buffer Concentrate flow rate percentages







Integrated BID vs. Stand-alone buffer dilution systems; advantages either way

YMC also design stand-alone systems for buffer and process solution preparation. Either way, integrated or stand-alone, these systems offer significant advantages to the user. Reference the table to the right for a brief comparison of the integrated and stand-alone approaches.



Figure 7. The LEWA ecodos pump has 4 diaphragm layers providing back-up in the unusual case one fails during operation. Additionally, a rupture sensor alerts the operator with adequate warning to avoid any leakage of fluid beyond the back-up membranes.



Figure 8. The system achieves extraordinary precision and accuracy due to unique fluid engineering design and exclusive LEWA intellidrive technology. The servo motor controls the buffer flow with digital precision and allows the system to achieve dilution factors of 1:150.

Table. Integrated BID vs stand-alone dilution system

	BID integrated on EcoPrime LPLC	Stand-alone YMC dilution system	
Number of process unit operations served by unit	One (LPLC)	Multiple (e.g. TFF, LPLC 1, 2)	
Cost to purchase	Nominal cost for standard option	Cost of new system	
Flow control metering of buffers	Yes	Yes	
Reduction in tanks	Significant	Very significant	
Ability to handle complex and multiple buffers	Good	Excellent	
Dilution factor/flow error	50:1 / < 0.5%	50:1 / < 0.5%	





The future provides broader solutions for our customers

The innovation and growth of this and other EcoPrime product lines has attracted the attention of leading technology suppliers and users. YMC Co., Ltd. assumed all rights and production for the EcoPrime suite of systems in late 2018 from LEWA-Nikkiso America, Inc. This acquisition brings a broad spectrum of chromatographic resins, and columns ideal for large and small molecule purification. More about this new chapter for EcoPrime at <u>www.ymcpt.com</u>.

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

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