Introduction

Enhanced 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, each buffer is supplied to the system at a concentration greater than the required buffer concentration for the process step and then diluted. Dilution factors up to 150:1 can be achieved with the EcoPrime LPLC with enhanced BID.

While the two concentrated buffers are being supplied, the third pump delivers a diluent solution (typically purified water or water for injection [WFI]), diluting the buffers to the precise 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 manual buffer preparation and the space typically used for storing ready-to-use buffers.

Buffer Considerations

Care must be taken to ensure that the buffer preparations are not so concentrated that they precipitate out of solution in the system prior to dilution. 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 equipment construction should also be considered. For example, high sodium chloride (NaCl) concentrations may cause pitting and corrosion to the 316 L stainless steel (SS) product contact components if the contact time is unusually long or the solution remains stagnant in the system. It is good practice to adequately flush the system with water after using aggressive buffer solutions.

One 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 integrity.

Configuring BID With EcoPrime Software

EcoPrime LPLC software has two different methods for programming a dilution: (1) fixed percentage; or (2) buffer concentration factor:

The fixed percentage allows the user to specify the percentage of the total flow rate that is to be supplied by the dilution pump.

The 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 while diluting two concentrates, buffer A (channel A) and buffer B (channel B) must have the same supply and target concentration factors of the concentrate streams.
concentration factors. A single supply and target concentration factor is used for both concentrate streams (with the diluent solution from channel C). The software does not allow the user to specify individual concentrates and target concentration factors.

Both methods, fixed percentage and buffer concentration factor, allow the same concentrate to be used at different concentrations in different steps in a recipe.

EXAMPLE 1: Isocratic Step Elution With Enhanced BID

In this example, BID is used to dilute two buffers (factor of 1:10) for an isocratic step elution. The total flow rate is 5.0 L/min.

Channel C, the diluent pump, will deliver 90% of the total flow (4.5 L/min) to dilute the two buffers (A and B). When configuring the percentages of concentrate buffers, together they must equal the balance of the total flow (10% or 0.5 L/min). Refer to Table 1 for flow rates, and buffer and diluent percentages.

**Software Configuration**

To define the concentrate isocratic step percentages, buffers A and B are always defined in terms of the percentage of the total flow rate (Figure 2, following page).

EXAMPLE 2: Gradient Elution With Enhanced BID

In this example, BID is used to dilute two buffers (factor of 1:10) for a three-step gradient elution that includes one linear segment followed by two isocratic steps. The total flow rate is 5.0 L/min (Table 2, following page).

**TABLE 1.** 1:10 dilution, isocratic step elution.

<table>
<thead>
<tr>
<th>Step #</th>
<th>Channel A (buffer A)</th>
<th>Channel B (buffer B)</th>
<th>Channel C (diluent)</th>
<th>Total Flow % and Rate</th>
<th>Channel A Ratio to Channel A/B Flow</th>
<th>Channel B Ratio to Channel A/B Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10%, 0.5 L/min</td>
<td>0%, 0 L/min</td>
<td>90%, 4.5 L/min</td>
<td>100%, 5.0 L/min</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>8.5%, 0.425 L/min</td>
<td>1.5%, 0.075 L/min</td>
<td>90%, 4.5 L/min</td>
<td>100%, 5.0 L/min</td>
<td>85%</td>
<td>15%</td>
</tr>
<tr>
<td>3</td>
<td>5.0%, 0.25 L/min</td>
<td>5.0%, 0.25 L/min</td>
<td>90%, 4.5 L/min</td>
<td>100%, 5.0 L/min</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>4</td>
<td>1.5%, 0.075 L/min</td>
<td>8.5%, 0.425 L/min</td>
<td>90%, 4.5 L/min</td>
<td>100%, 5.0 L/min</td>
<td>15%</td>
<td>85%</td>
</tr>
<tr>
<td>5</td>
<td>0%, 0 L/min</td>
<td>10%, 0.5 L/min</td>
<td>90%, 4.5 L/min</td>
<td>100%, 5.0 L/min</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Analytical performance at pilot and production scale. And more.

LEWA EcoPrime® LPLC achieves accuracy and precision better than 99% with linear gradients from 1 to 99%. Add to that the versatility of our on-board buffer in-line dilution system, and you’ve got all the makings of a real chromatography wunderkind.

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Overachiever.
FIGURE 3. Gradient parameters screen showing fixed % dilution method with 90% channel C (diluent) and gradient buffer B (channel B) percentages in three-step gradient elution.

Channel C, the diluent pump, will deliver 90% of the total flow (4.5 L/min) to dilute the two buffers (A and B).

Software Configuration

When configuring the percentages of concentrate buffers, the gradient (linear and step) starting and ending percentages are always defined in terms of the percentage of the gradient flow rate of the system (Figure 3). Buffer B is the specified gradient buffer and the EcoPrime software calculates the percentage of buffer A.

TABLE 2. 1:10 dilution, linear and step gradients.

<table>
<thead>
<tr>
<th>Gradient Change #</th>
<th>Channel A (calculated)</th>
<th>Channel B (Buffer B)</th>
<th>Channel C (Diluent)</th>
<th>Totals for Channels A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starting</td>
<td>Ending</td>
<td>Starting</td>
<td>Ending</td>
</tr>
<tr>
<td>1</td>
<td>100%, 0.5 L/min</td>
<td>50%, 0.25 L/min</td>
<td>0%, 0L/min</td>
<td>50%, 0.25 L/min</td>
</tr>
<tr>
<td>2</td>
<td>50%, 0.25 L/min</td>
<td>25%, 0.125 L/min</td>
<td>50%, 0.25 L/min</td>
<td>75%, 0.375 L/min</td>
</tr>
<tr>
<td>3</td>
<td>25%, 0.125 L/min</td>
<td>0%, 0L/min</td>
<td>75%, 0.375 L/min</td>
<td>100%, 0.5 L/min</td>
</tr>
</tbody>
</table>

FIGURE 2. Isocratic step parameters screen showing concentrate buffer percentages.
The EcoPrime LPLC system (Figure 4), with an enhanced BID option (Figure 5), utilizes LEWA’s digital fluid control with state-of-the-art pumps and exclusive digital pump control technology (Figure 6), providing the highest volumetric flow precision and accuracy available today. The precise and accurate flow of the LEWA ecodos®® metering pumps (Figure 7) used in the system enable dilution factors up to 150:1 with volumetric flow error of <0.5% (Figure 8).

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

**FIGURE 4 (right).** EcoPrime LPLC select features.

**FIGURE 5 (below).** The enhanced BID option on the EcoPrime LPLC consists of a third pump incorporated seamlessly into the system with no increase in footprint.
Low-Pressure Liquid Chromatography Using the EcoPrime With Enhanced Buffer In-Line Dilution

**FIGURE 6 (left).** The LEWA hygienic ecodos pump has four diaphragm layers providing backup in the unlikely case that one fails during operation. Additionally, a rupture sensor alerts the operator with adequate warning to avoid any leakage of fluid beyond the backup membranes.

**FIGURE 7 (right).** The system achieves extraordinary precision and accuracy due to its unique fluid engineering design and exclusive LEWA intellidrive technology. The servo motor controls the buffer flow with digital controls and allows the system to achieve dilution factors of 1:150.

**FIGURE 8 (above).** EcoPrime LPLC with enhanced BID achieves volumetric flow accuracy >0.5% for dilution factors of 10×, 25×, 50×, 100×, and 150×.

- **Buffer Concentrates:** Channels A and B; and the diluent: channel C.
- **Data Shown:** Channel A (channel B data not shown).
- **Flow Rate Percentages:** Determined by measuring tryptophan UV signal at 280 nm. The total system flow rate is 9.00 L/m.
- **Target and Measured Concentration (%)**: X-axis (left)—The target and actual channel A buffer concentrate flow rate percentages.
- **Error (%)**: X-axis (right)—The target and actual channel A buffer concentrate flow rate percentages.
- **Target Channel A Concentration (%)**: The target channel A buffer concentrate flow rate percentage.
- **Actual Target A Concentration (%)**: The actual channel A buffer concentrate flow rate percentage.
- **Channel A Concentration Error (%)**: The target and actual channel A buffer concentrate flow rate percentages.
Low-Pressure Liquid Chromatography Using the EcoPrime With Enhanced Buffer In-Line Dilution

**Integrated BID vs. Stand-Alone Buffer Dilution Systems: Both Have Their Advantages**

LEWA also designs stand-alone systems for buffer and process solution preparation. Whether integrated or stand-alone, these systems offer significant advantages to the user. Table 3 shows a brief comparison of the integrated and stand-alone approaches.

<table>
<thead>
<tr>
<th>Considerations</th>
<th>BID Integrated on LEWA EcoPrime LPLC System</th>
<th>Stand-Alone LEWA Buffer Dilution System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of process unit operations served by system</td>
<td>One (LPLC)</td>
<td>Multiple (e.g., TFF, LPLC 1, LPLC2, etc.)</td>
</tr>
<tr>
<td>Equipment and operational costs</td>
<td>Economical operation because it is built-in</td>
<td>Must be purchased individually</td>
</tr>
<tr>
<td>Capability for flow control metering of buffers</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reduction gained in tank farm</td>
<td>Significant</td>
<td>Very significant</td>
</tr>
<tr>
<td>Ability to handle complex and multiple buffer regimes</td>
<td>Good</td>
<td>Excellent</td>
</tr>
<tr>
<td>Dilution factor/flow error</td>
<td>150:1/&lt;0.5%</td>
<td>150:1/&lt;0.5%</td>
</tr>
</tbody>
</table>

**Conclusion**

As a result of the exceptional flow rate performance with the EcoPrime system, 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 LEWA approach, using the volumetric flow performance of our metering pumps to blend buffer concentrates and diluents, you eliminate process variables, assuring better control. The result is a capable and simplified system that can be easily validated:

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

**About the Authors**

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