ABSTRACT
Process-scale chromatography plays an important role in the purification of pharmaceutical products derived from biotechnology processes. This unit operation can be manually intensive with lengthy cycle times. This poster discusses how on-line HPLC is a tool that can be utilized to measure the product purity in near real time which can reduce product variability, increase process efficiency, and enable automation and control.

INTRODUCTION
Pharmaceutical products derived from biotechnology based processes frequently utilize process chromatography to isolate and purify the product of interest. Knowing when the product of interest elutes from these process-scale columns, at sufficient purity to be forward processed, can be difficult because these separations rarely provide baseline separation of the product of interest from other components in the process stream. Determining where the product elution occurs generally involves collecting the column eluent stream in small volumes that are sampled and analyzed in the laboratory utilizing higher-resolution chromatographic techniques such as reversed-phase chromatography. This process of eluent fractionation and off-line chromatographic analysis is performed in the process development area in order to generate sufficient process understanding to enable prediction of when the product elutes from the process-scale chromatography column. The process of column fractionation is often continued when the product moves into manufacturing. Fractionation of the column eluent has several negative issues associated with it including:

- Labor intensive
- Opportunities for errors to occur
- Opportunities for product contamination
- Increased process cycle time caused by time to obtain off-line assays
- Storage required for work in process (the column fractions)
- Degradation of product caused by the delays
- Reduced production capacity due to long cycle times
- Difficulty automating processing due to manual handling of fractions

Due to the many negative issues associated with column fractionation, companies strive to eliminate this process operation as quickly as possible. Options to eliminate fractionation include:

1. **Collection of product based on column eluent volume.**
   In this approach, the eluent volume where the product of sufficient purity is expected to elute is determined from historical data, and the start collection and stop collection setpoints are set. An in-line flow meter is used to measure the eluent volume to control the collection of the product of interest as it elutes from the process chromatography column. The issue with this approach is that it is totally dependent on the reproducibility of the column elution profile, which is impacted by column loading, purity of the starting material, column packing, and column gradient generation. Even with feedback control of the gradient generation, it can be challenging to maintain a reproducible purity of the recovered product utilizing this approach. This variability leads to conservative collection setpoints that result in lower product yields.

2. **Collection of product based on column eluent volume and optical density.**
   In this approach, an in-line UV sensor set to 280 nm is used to measure the absorbance from the product's peptide bonds. Since this is a nonproduct specific measurement (i.e., any peptide in the stream will generate a signal) the OD sensor's output is examined in combination with the in-line flow meter to gain an additional level of resolution. That is, the product is only collected when the OD at 280 nm exceeds the OD setpoint and falls within the volume setpoint window. This imparts greater selectivity than using the eluent volume approach alone, but is still dependent on the reproducibility of the column's elution profile. Although the variability is less with this approach, the lack of a high level of selectivity still leads to conservative collection setpoints that negatively impact product yields.

3. **Measurement of product purity by on-line HPLC.**
   The critical quality attribute of the product is its purity. Meeting a specified purity value is the criterion that determines if the process chromatography step has been successful, and if the in-process material is suitable for forward processing. By transferring the specificity of HPLC to an on-line analyzer, it is possible to directly measure the critical quality attributes in near real time, thus allowing the process decision (i.e., when to start and stop collection of the product eluting from the process chromatography column) to be based on the critical quality attribute rather than being based on a surrogate measurement that is impacted by the process variability.
AUTOMATING PROCESS CHROMATOGRAPHY USING ON-LINE HPLC

Figure 1 is a diagram of how the product purity data from the on-line HPLC is transmitted to the Distributed Control System (DCS), where it is compared to the product purity setpoints. From these data the DCS will determine when to start and stop the collection of the product based on the critical quality attribute, which is the product's purity value. The diagram also shows the critical operating parameters for the unit operation that are also transmitted to the DCS, where feedback control of these parameters is performed to minimize process variability.

In this operation, the on-line HPLC sends the processed chromatographic data to the DCS after completing the analysis of each sample injection. The product purity data from the on-line HPLC is compared to the product purity setpoint in the DCS. If the product purity is less than the product purity setpoint, the DCS sends a signal to the three-way valve in the eluent stream to divert the eluent stream to waste. If the product purity is greater than the product purity setpoint, the DCS sends a signal to the three-way valve in the eluent stream to divert the eluent stream to the product collection tank. By basing the mainstream pooling decision on a direct measurement of the product purity, product variability is minimized. The dependency of the product's purity on the operation of the process chromatography column is significantly reduced when compared to a collection of product pool based on elution volume or a combination of elution volume and optical density.

Most biotech processes involve multiple column chromatography steps to achieve adequate purification of the biosynthetic origin product. Using on-line HPLC, Eli Lilly and Company was able to combine two process-scale chromatography steps into a single, automated process. This process is explained in Figures 2 through 7.

Figure 2 shows the two process chromatography columns combined via several process valves to enable the columns to be operated either in parallel with one another (two independent columns) or in series. An on-line HPLC analyzer is also added to allow the column eluent stream to be sampled and automatically analyzed to determine the product's purity. This data is used by the DCS to determine the appropriate position of the process valves (to operate the process columns either in parallel or series) and to divert the eluent stream to waste or to the product collection tank.

Figure 3 shows the two process-scale columns operated in parallel mode. The on-line HPLC is automatically analyzing the column 1 eluent stream and sending the data to the DCS system where it is compared to the product purity setpoint. Since only frontside impurities are being found in the eluent stream (i.e., no product is present) the stream is automatically diverted to waste with the two process columns being maintained in parallel mode.
In the next step, the backside impurities are detected eluting from Column 1. The DCS automatically adjusts the process valves, shown in Figure 5, so that the two process columns are operating in parallel, allowing the backside impurities from Column 1 to be eluted to waste. Repositioning the process valves also allows the same on-line HPLC that was monitoring the eluent from Column 1 to now monitor the eluent stream from Column 2. The frontside impurities are detected eluting from Column 2 and are automatically diverted to waste by the DCS.

The high-purity product is detected eluting from Column 2 by the on-line HPLC. When this occurs, the DCS repositions the valves, as shown in Figure 6, to allow the product to be automatically collected in the product hold tank. With the process valves in this position, the columns are operating independently of one another (parallel mode). This allows Column 1 to undergo regeneration while Column 2 is being eluted. Performing these two operations simultaneously further reduces the overall process cycle time.
CONCLUSIONS

Using on-line HPLC to monitor the eluent from process-scale chromatography columns allows the volume of the eluent stream that is collected to be based on the critical quality attribute, which is product purity. Measuring the critical quality attribute and using this to adjust the eluent collection point allows the process to flex to compensate for variability in the separation. Adjusting the eluent collection points allows the process to compensate for the chromatography variability rather than have it show up as increased variability in the product purity. In addition to reducing product variability, using on-line HPLC also increases product yield, enables the use of increased levels of automation, and reduces overall cycle time. In the example described in this poster, the process throughput was increased tenfold, allowing production to be increased from 6 to 8 kg of product per campaign to 60 to 80 kg of product per campaign.

ACKNOWLEDGEMENT

The author retired from Eli Lilly and Company in 2005 where he was involved in the implementation of the on-line HPLC example described in this poster. He would like to thank the company for allowing him to utilize their process example to demonstrate the value of on-line HPLC.