Liquid Chromatography as a Process Analytical Technology for On-Line Monitoring of Biotech Processes

The FDA's PAT initiative has generated a great deal of interest in in-line, on-line, and at-line analytical technologies. These tools can be used to increase process understanding, decrease variability, and increase efficiency through timelier measurement of critical-to-quality process and product attributes. Implementing this type of technology in biotech processes has proven to be a much greater challenge compared with its use in small-molecule processes. This is due in great part to the complexity of typical biotech processes, where the product may start as a minor component (<1%) of a bioreactor containing thousands of molecules, and where product functionality depends critically on primary, secondary, and tertiary molecular structure. On-line liquid chromatography has proven its ability to meet these challenges of process analysis in the biotech industry.

Liquid chromatography (LC) has become the major analytical technique used in the pharmaceutical/biopharmaceutical laboratory to monitor product quality. The major reasons for the high use of this technology are its applicability to a broad variety of molecules, broad measurement range, and resolution. Resolution is a particularly valuable attribute in the highly complex matrices typically encountered in biotech processes.

The Dionex DX-800 Process Analyzer provides automated sample preparation capability, isocratic or quaternary gradient pump options, and a choice of standard detector options (UV-VIS, conductivity, or pulsed amperometric) that make it possible to move many IC and HPLC methods from the laboratory, to the process area, where information about critical-to-quality product and/or raw material attributes can be generated automatically without operator intervention. The OLE for Process Control (OPC) server feature of the analyzer’s Chromelon® PA software enables sharing of the information generated by the DX-800 with plant IT systems for use in implementing advanced process automation and control. The increased amount and frequency of information generated by on-line LC can prove highly valuable.

For example, the makeup of bioreactor cell culture media can significantly impact the ability and efficiency of cells to produce the desired product. The DX-800 on-line LC system enables process development scientists to measure media components such as amino acids, carbohydrates, and inorganic anions and organic acids, on line without pre- or postcolumn derivatization (1–3). The information generated by the DX-800 can be invaluable in deepening understanding of how these components are affecting product expression across cell lines, as described by Genentech(4, 5).

On-line LC also has a role in downstream operations. Eli Lilly and Company use it to provide on-line analysis of purity as the product elutes from process-scale purification columns (6). The on-line HPLC information automatically controls the collection of peptide products based on direct measurement of product purity. On-line HPLC enabled the combination of two process-scale chromatography steps into a single, semicontinuous operation that increased process throughput 10x (7).

On-line HPLC can also help determine the optimum endpoint of a process-scale reaction — for example, one that involves the enzymatic conversion of a peptide intermediate to the final form used in the drug product (8). By applying on-line HPLC to monitor the progress of the enzymatic reaction, Eli Lilly can stop the reaction at the optimum endpoint, where yield is maximized, the formation of related substances is minimized, and the purity and yield of the process intermediate are more consistent.

LC FOR PROCESS MONITORING

Liquid chromatography has proven to be a mainstay technology for monitoring biotech product quality. Moving this technology from the laboratory to the production floor enables the application of LC to near–real-time analysis of process streams, leading to increased process understanding, decreased variability, and improved efficiency through increased yields, purity, and throughput.

REFERENCES

1. Determination of Amino Acids in Cell Cultures and Fermentation Broths. Application Note 150, Dionex Corporation, Sunnyvale, CA.
2. Determination of Carbohydrates, Alcohols, and Glycols in Fermentation Broths. Application Note 122, Dionex Corporation, Sunnyvale, CA.

Rick E. Cooley is manager of the Process Analytics Center of Excellence for Dionex Corporation, Sunnyvale, CA, and formerly of Eli Lilly and Company (retired); www.dionex.com.