

Application News

Liquid Chromatography

Using the Shimadzu HPLC System in the Fuel-Grade Ethanol Production Laboratory

Liquid Chromatography (LC) is any chromatographic procedure in which the moving phase is a liquid. In most cases, a stationary phase is also necessary. High Performance Liquid Chromatography (HPLC) has become the most widely applicable method of chromatographic analysis. Through use of highly efficient column materials (the stationary phase), pumping systems that provide a consistent flow of the mobile phase, and detection components designed to accommodate the needs of the analysis, HPLC is now a commonly used technique for monitoring the progress of the fermentation in the ethanol production laboratory.

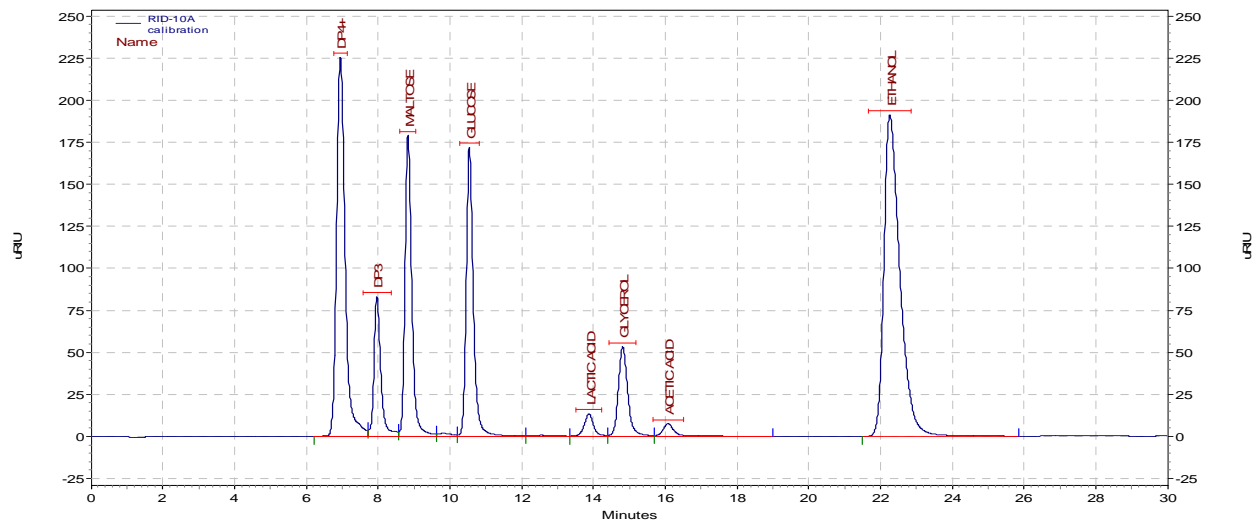
LC makes use of both the stationary phase and the solvent (mobile phase) to effect control over the separation. The separation of the molecules in the sample is dependent on many types of interactions that occur between the column phase, mobile phase, and sample molecules as they move toward the detector.

In the case of the ethanol production plant, the HPLC system is typically used to profile the carbohydrate, alcohol, and organic acid content of the fermentation broth. The column technology that performs this analysis uses ion exchange, ion exclusion, and size exclusion technologies. In order to make this column work effectively in this analysis, the mobile phase is a dilute solution of sulfuric acid (0.002 – 0.005 N).

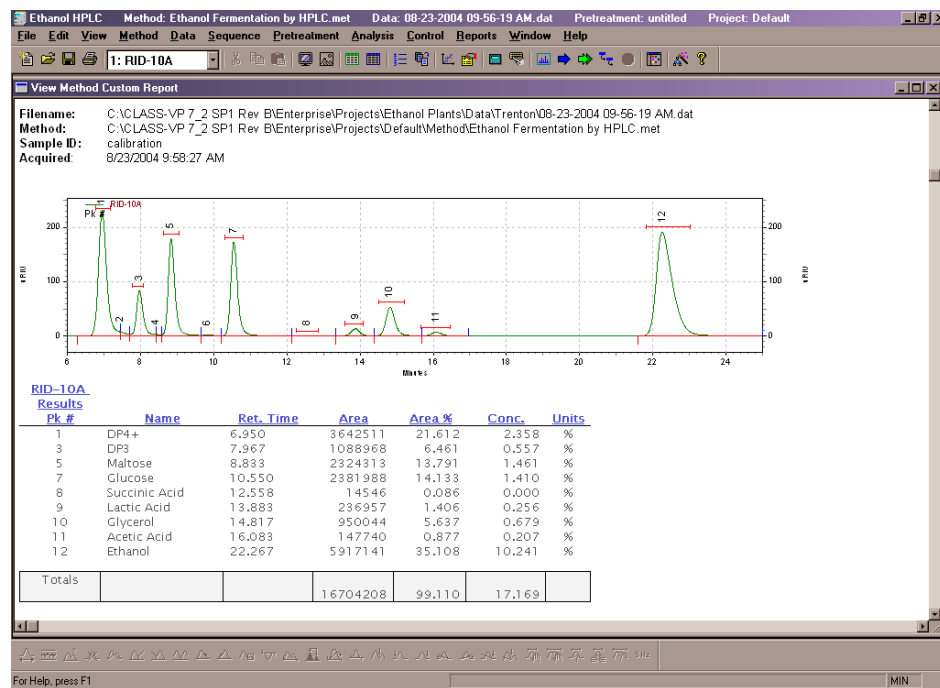
The HPLC equipment that is used to perform the fermentation analysis is comprised of several components that make the analysis easy and reliable. The necessary components are 1) the mobile phase, as previously discussed, 2) an isocratic pumping system, 3) an autosampler for sample introduction, 4) the analytical column (as previously discussed), 5) the refractive index detector, and 6) the data acquisition system. Most systems also include a mobile phase degasser to maintain a bubble-free mobile phase delivery, and a column heater. Lastly, many systems utilize a sample cooler to minimize ongoing sample metabolism.

Fermentation broth samples are obtained during specific time points in the process. The analysis of the fermentation broth is intended to monitor the breakdown of the starch molecules into glucose, then the conversion into ethanol, following typical Krebs cycle dynamics. The fermentation can go too far if the ethanol begins to be converted into acetic acid.

Injection of a filtered broth sample is carried through the column to the detector by the dilute acid mobile phase. A typical chromatogram is shown below.



Calibration of the HPLC system by use of a standard solution of the components of interest will allow the user to obtain, directly, as weight percent for the analytes of the broth samples. This data can be used to evaluate the progress of the fermentation and what intervention may be necessary at a future time to maximize the production of ethanol and minimize the production of further oxidation products.



Typical Instrumentation: HPLC Hardware Controller: CBM-20A or SCL-10Avp; HPLC Pump: LC-20AT; Mobile Phase Degasser: DGU-20A3; Autoinjector: SIL-10AF; Column Oven: CTO-20A; Detector: RID-10A; Shimadzu CLASS-VP Software; Column: Phenomenex Rezex ROA, 300 mm x 7.8 mm; Mobile Phase: 0.005 N Sulfuric Acid; Flow Rate: 0.600 mL/min; Column Temperature: 65°C.