Chromatography Techniques
Product Innovation for Chromatographers

DOE, HPLC Validate Corn Ethanol Measurement Technology

The simplicity of design of experiments, combined with advanced HPLC technology, led the way to a new, successful sample preparation method.

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An industrial equipment supplier wanted to sell an existing product to companies producing ethanol from corn. Anecdotal evidence showed the device increased ethanol yield. But, before putting the device on the market the company wanted to find the best operating conditions and determine what performance its product could deliver for ethanol producers. The first step was finding the right measurement method and a way to preserve samples so they would not deteriorate during shipment from plant to lab. After an accurate measurement method had been identified, the next step was running a number of tests to get an idea of which factors affected sample preservation. Finally, researchers performed a design of experiments (DOE) that quantified the effects of these factors, singly and in combination, on sample preservation. The sample preservation method identified by the DOE functioned well on hundreds of samples at ethanol plants across the Midwest.

Stites & Associates, LLC, (SALLC) is a technology development and improvement company working in a wide variety of energy applications. The main activities of SALLC include: setting up labs and experiments, evaluating data, evaluating lab operations, and evaluating and improving technologies. Stites & Associates operates a research laboratory in Brighton, Colo., that performs gas chromatography mass spectroscopy (GCMS), cyclic voltammetry and optical microscopy.

"I have found that by combining excellent analytical work with diligent research and outside-the-box thinking it is possible to not only evaluate existing technology but to gain insight into the best ways to try to improve a technology," said Ron Stites, Managing Member of SALLC. "Often this combination results in learning how to make non-obvious improvements that can result in real breakthroughs." Stites is the former director of research for Range Fuels, holds three patents in alternative fuels and has been an independent consultant since 1996.

In this application, the industrial equipment supplier contracted with SALLC to develop a method for measuring how well the supplier's product improved performance to the corn ethanol market. The first step in producing ethanol from corn is soaking the grain in water to make it easier to separate the components on the corn kernel. The corn is milled to produce a corn flour that is slurried with water and the heat-stable enzyme α-amylase is added. This slurry is cooked in a process known as liquefaction that uses heat and mechanical shear to break apart starch molecules into small fractions. After liquefaction, the corn slurry, now called corn mash, is cooled to approximately 30 C and glucoamylase enzyme is added to complete the breakdown of starch into glucose. In the fermentation step, yeast is added to the corn mash to convert the simple sugars to ethanol.

The breakdown of starch to glucose takes place in two steps, from starch to maltrodextrins and then from maltodextrins to glucose. The equipment supplier hypothesized that its product could speed up the breakdown of starch to glucose while using less enzymes. The goal was also to minimize "burning," a phenomena that occurs when shorter-chain sugars react together and with proteins to form polymers that yeast cannot digest. In fact, some are even toxic to yeast. This process is monitored by analysis of the degree of polymerization (DP) of the maltodextrins. But measurement of maltrodextrins is challenging. The main difficulty is the analysis requires specialized techniques that are not available in the plant so samples must be sent to the lab. But the samples are not stable so the breakdown of starch continues during transport.

Alternative routes

There are about 200 ethanol plants in the Midwest and samples would need to be shipped from any plant that tried the new product to the SALLC lab. Stites recognized that he needed to find a way to stop the reaction for 72 hours until the sample could be shipped to the lab and tested. Samples from the actual production process were too inconsistent to use for developing the sample preservation process. Stites found that spray-dried maltodextrin provides a consistent starting material to evaluate methods of stopping the reaction. Stites researched the enzymes and found they did not work well at low pH and low temperatures. So, he lowered the pH by adding sulfuric acid, cooled the samples and held them at shipping times from 24 to 72 hours before testing. A pH of 2 appeared to prevent the enzymes from working. Stites reviewed published literature and found a new method based on high performance liquid chromatography (HPLC) that could accurately measure maltodextrins. He set up equipment in the SALLC lab to perform the method. The HPLC method easily detected changes in sugar concentration (Figure 1).

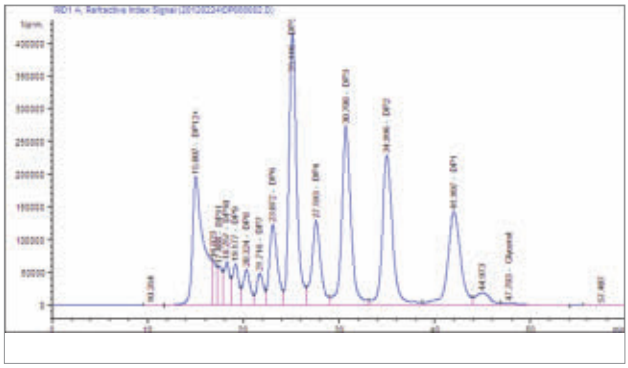

Figure 1: HPLC results for DP measurements of maltodextrin

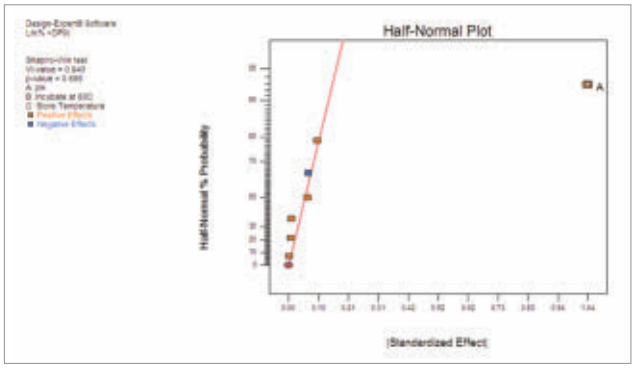

Figure 2: Half-normal plot demonstrates that DOE results are significant

With this preliminary work out of the way, the actual DOE was relatively simple. Stites turned to Design-Expert software (Stat-Ease, Inc.), which walks users through the process of designing and running an experiment and evaluating the results.

The factors, runs and results for the DOE are shown in Table 1. The %<DP9 in the sample at the start of the experiment is 25.00%. The response %<DP9 shows how much this value increased during the experiment. The results show that pH is the most important factor and that the other factors all had minimal effects, although incubation at 60 C made things slightly worse by speeding up the reaction. The best results were provided by pH adjustment to 2.0 and storage at 3 C with the DP increasing to only 25.04% after 48 hours. This is less than the standard deviation of the HPLC method and this method can be easily performed in the field.

Next, Stites addressed the question of whether or not these results are significant or could have been achieved by chance. Factorial design analysis uses the half-normal plot to identify significant effects. The orange and blue rectangles on the half-normal plot in Figure 2 show the effects, positive and negative respectively, and the position of these rectangles reflect the relative size. The further the factor effects are from the line near zero, the more likely they are to be significant. In this case, the factor effects of variable A, which is pH, are much greater than the variation between the insignificant effects, demonstrating the statistical significance of the experiment.

The method of run 6 was selected and worked very well from the beginning for samples from the slurry and liquefaction steps. Some strange results were seen with fermentation samples—most of the carbohydrates were gone. It was discovered that the sulfuric acid was interfering with the HPLC measurements. The method was modified to remove the sulfuric acid before analysis by treatment with barium hydroxide and filtering. Since this modification was made, the method has worked with samples from all three steps.

Hundreds of samples were shipped from ethanol plants around the Midwest and used to evaluate the performance of the new product. Some plants found significantly better results and others did not see significant improvements.

"However, the DOE was clearly successful in its ability to identify and validate a measurement method that has enabled us to accurately evaluate the performance of the new product in a large number of plants under a wide range of operating conditions," said Stites. LT

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A:pH	B:Incubate at 60C	C:Store Temperature	% <DP9
Units		pH		C	%
3	1	2	No	3	29.22%
6	2	5.5	Yes	25	77.17%
8	3	5.5	No	25	77.45%
5	4	2	Yes	25	24.49%
1	5	2	Yes	3	25.20%
7	6	2	No	25	25.04%
4	7	5.5	No	3	64.99%
2	8	5.5	Yes	3	74.31%

Table 1: DOE used to explore sample preservation methods