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## Using Accelerated Solvent Extraction (ASE) in Alternative Fuel Research

### **INTRODUCTION**

As global interest in alternate fuel sources increases, many laboratories are researching effective ways to test, develop, and produce fuel from renewable energy sources. One of these is the production of alcohol from biomass, called bioalcohol. Alcohol produced from biomass has several benefits over fossil fuels because a) it is produced from crops, a renewable energy source, and b) when burned, it causes little environmental pollution. As bioalcohol is produced from biomass via sugar fermentation, it is necessary to research which types of plants produce the best yields of usable sugars.

The Dionex Accelerated Solvent Extraction (ASE<sup>®</sup>) systems provide a fast and efficient way to extract various biomass samples for sugar analysis to determine sample viability for alcohol production. The new ASE 150 and 350 systems with the pH-hardened pathways provide additional benefits to the method by allowing pre-hydrolyzed biomass samples or those with acidified solvents to be extracted. This allows samples to be hydrolyzed in the extraction cell.<sup>1</sup> This application note describes methods for the extraction of sugars from biomass samples using acidified solvents (in-cell hydrolysis) and extracting pre-hydrolyzed samples.<sup>2</sup>

### **EQUIPMENT**

Dionex ASE 350 Accelerated Solvent Extractor  
(P/N 057794)  
Dionium™ Extraction Cells 66 mL (P/N 068102)  
Collection Bottles 250 mL (P/N 056284)  
Glass Fiber Filters (P/N 056781)  
Ottawa Sand (Fisher Scientific)  
Standard Laboratory Sample Evaporation System  
Standard Laboratory Grinder or Mill

### **REAGENTS**

HPLC Grade Water (Fisher Scientific)  
H<sub>2</sub>SO<sub>4</sub> 8 M (Sigma-Aldrich)

### **SAMPLE PREP**

Grind raw sample material using a standard laboratory grinder or mill.

### **Sample Prep for Acidified Solvent Extraction (In-Cell Hydrolysis):**

Weigh approximately 1.0–10.0 g of ground sample into a tared 66 mL Dionium extraction cell containing a glass fiber filter. It is important not to compact the sample into the cell as a tightly compacted sample will not hydrolyze well. Ottawa Sand can be added to the cell to take up any dead volume. This will help reduce the amount of solvent used per extraction. Weigh the appropriate number of 250 mL collection vials and place them onto the ASE system.

### Sample Prep for Pre-Hydrolyzed Samples

Weigh approximately 2 g of sample and hydrolyze using 0.5% H<sub>2</sub>SO<sub>4</sub> at 200 °C. Transfer the acidified sample mixture to a 66 mL Dionium extraction cell containing a glass fiber filter. Weigh the appropriate number of 250 mL collection bottles and place them onto the ASE system.

### ASE CONDITIONS

#### ASE Conditions using Acidified Solvent

Pressure: 1500 psi\*  
Temperature: 140–150 °C<sup>a</sup>  
Solvent: 0.1 M H<sub>2</sub>SO<sub>4</sub> solution with HPLC water  
Static Time: 5 min  
Static Cycles: 3  
Flush: 10%  
Purge: 120 s

*\*Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications*

<sup>a</sup>Tests concluded that an extraction temperature of no higher than 150 °C was optimal for the corn stover samples. Other biomass sample matrices may vary and could require an extraction temperature lower than 150 °C.

#### ASE Conditions for Pre-Hydrolyzed Samples<sup>2</sup>

Pressure: 1500 psi  
Temperature: 100 °C  
Solvent: HPLC water  
Static Time: 7 min  
Static Cycles: 3  
Flush: 50%  
Purge: 120 s

### EXTRACTION

Place the extraction cells containing the samples onto the ASE system cell tray. Create an extraction method based on the desired method listed above. Once the extractions are completed, prepare the extracts according to the analytical method needed. For gravimetric determination, evaporate the solvent to dryness using a standard laboratory sample evaporation system and weigh the collection vials to determine % residue.

$(\text{Weight of evaporated vial} - \text{tared weight of vial}) / \text{sample weight} \times 100$ .

If sample is to be analyzed chromatographically for sugar content, then the appropriate volume adjustment should be made before injection into the chromatographic system.

## RESULTS AND DISCUSSION

### Acidified Solvent Extraction (In-Cell Hydrolysis)

Corn stover<sup>b</sup> samples were extracted for the quantifiable determination of sugars used for ethanol production. Two different extraction temperatures were examined for determining the best method (Table 1). Results showed that the samples extracted at 190 °C produced more total extractables. However, HPLC analysis showed that this higher temperature caused a breakdown of the sugars. The higher temperature was too aggressive and caused an extraction of unwanted coextractables resulting in the increased final weight. Analysis of the extracts at 150 °C showed no breakdown of sugars and no increase in unwanted coextractables.

<sup>b</sup>Corn stover consists of the leaves and stalks of maize plants left in a field after harvest and includes the residue stalk, the leaf, husk, and cob.

**Table 1. Corn Stover Extracted with Acidified Solvent**

Extraction Temperature	% Total Extractables
150 °C	48.7
190 °C	87.3

### Pre-Hydrolyzed Samples

Two different concentrations of acidic solvents were examined for hydrolysis of switchgrass samples to determine the optimal concentration for recovery of glucose (Table 2). The HPLC analysis showed that for samples pretreated with 0.2% H<sub>2</sub>SO<sub>4</sub>, xylose was the predominant sugar present (Figure 1). Samples pretreated with 0.5% H<sub>2</sub>SO<sub>4</sub> had a significant increase in glucose levels (Figure 2). Table 3 lists the HPLC conditions.

**Table 2. Pre-Hydrolyzed Switchgrass Samples Extracted with HPLC-Grade Water**

Acidic Solvent Concentration	% Total Extractables
0.2% H <sub>2</sub> SO <sub>4</sub>	76.76
0.5% H <sub>2</sub> SO <sub>4</sub>	72.39

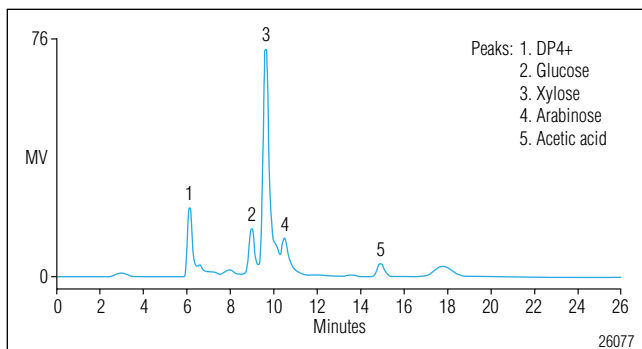


Figure 1. HPLC characterization of switchgrass pretreated with 0.2%  $H_2SO_4$

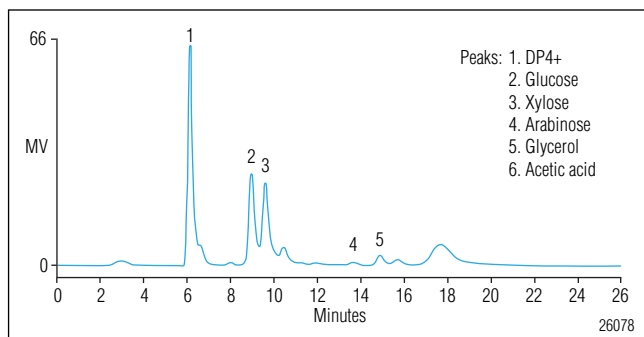


Figure 2. HPLC characterization of switchgrass pretreated with 0.5%  $H_2SO_4$

**Table 3. HPLC Conditions for Figures 1 and 2**

Column Type	Aminex <sup>®</sup> HPX-87H
Flow Rate	0.6 mL/min
Temperature	65 °C
Mobile Phase	0.0005 N $H_2SO_4$
Detector	RI
Run Time	26 min
Post Run Time	10 min

## CONCLUSIONS

The new pH-hardened pathways of the ASE 350 and 150 systems allow the extraction of samples that have been pretreated with acid, and also allows in-cell hydrolysis with acidified solvents. This new feature, along with the rapid extraction time and minimal solvent usage, provides users with a way to rapidly quantify biomass samples for biofuel research.

## ACKNOWLEDGEMENTS

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## LIST OF MANUFACTURERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, [www.fishersci.com](http://www.fishersci.com).

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, [www.sigmaaldrich.com](http://www.sigmaaldrich.com).

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1. *Accelerated Solvent Extraction ASE Systems* Brochure, LPN 2012-02, 2008. Dionex Corporation, Sunnyvale, CA.
2. Minnesota Chromatography Forum, Poster Presentation (SDSU) May 7, 2008.

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### Dionex Corporation

1228 Titan Way  
P.O. Box 3603  
Sunnyvale, CA  
94088-3603  
(408) 737-0700

### North America

U.S./Canada (847) 295-7500

### South America

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