Cellulose Hydrolysate Analysis by HPLC

Application Note

Biofuels

Authors

Stephen Ball, Linda Lloyd,
Keeley Mapp
Agilent Technologies, Inc.

Introduction

Cellulose is a polysaccharide consisting of a linear chain of several hundred to over ten thousand linked D-glucose units. It provides the structure of the cell wall in green plants. Cellulose can be hydrolyzed into its glucose units by treating it with concentrated acids at high temperature. Alternatively, enzymes such as the endo-acting cellulase break cellulose down into individual glucose units.

HPLC using an Agilent Hi-Plex Ca column analyzes the breakdown products of an enzymic digestion of cellulose.

Agilent Technologies

Experimental

An isocratic HPLC system was set up with a column block heater and an RI detector.

Conditions

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Mobile phase	100% DI H_2O
Flow rate	0.6 mL/min
Temperature	85 °C

Sample Preparation

A 10 mg/mL solution of cellulase (CAS 9012-54-8) in water was adjusted to an approximate pH of 4.5 with 0.01 M HCl. Ten milliliters of this solution were then added to 0.1 g of chromatography-grade cellulose (CAS 9004-34-6) in a 25 mL conical flask.

The contents of the flask were left in a water bath and heated to 40 °C for 24 hours, during which time 1 mL aliquots were extracted for analysis. Each sample and the liquid remaining after 24 hours were passed through a 0.45 µm syringe filter to remove any remaining cellulose from the sample (effectively preventing any further hydrolysis). All samples were stored in the freezer before analysis.

Twenty microliter injections were made of each sample to analyze for breakdown products.

Results

The following chromatograms track the levels of the sugars resulting from the enzymic hydrolysis of cellulose over time.

Aliquots were collected at 2 hours through the process, 19 hours (after being left overnight), 21 hours, and finally 24 hours.

Discussion

From an early stage in the process, two different sugar molecules begin to form in the solution: glucose and cellobiose.

Cellobiose is a disaccharide derived from the condensation of two glucose molecules linked in a ß (1 ⇒ 4) bond. This is a byproduct of the enzyme-catalyzed hydrolysis of cellulose.

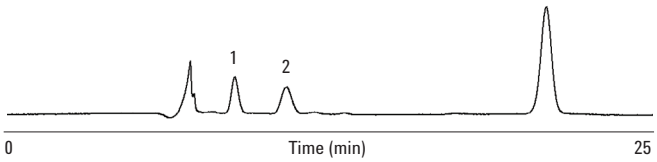
As the elapsed time increases, so does the concentration of cellobiose and glucose, indicating that increased numbers of cellulose chains are breaking down into smaller sugar units.

The additional peaks at the beginning and end of the chromatograms are likely to be additional side-products of the hydrolysis reaction or cellulase itself present in solution.

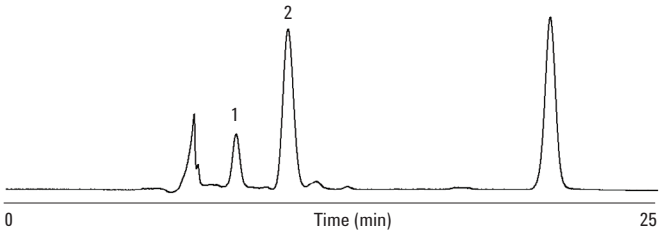
Peak identification (for all figures)

1. Glucose
2. Cellobiose

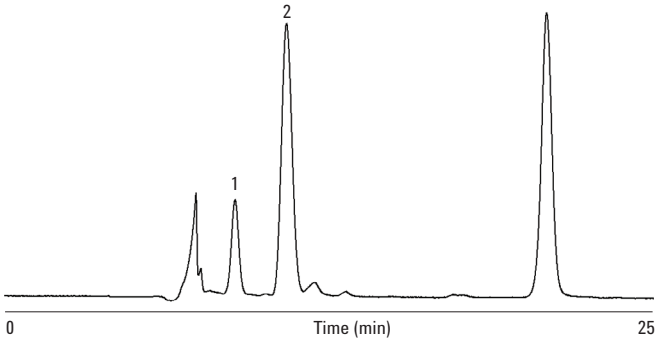
Reaction time: 2 hours



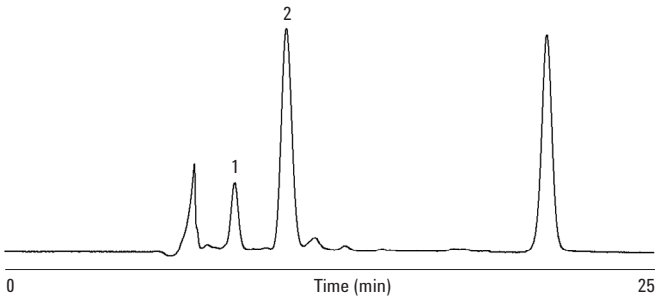
Reaction time: 19 hours



Reaction time: 21 hours



Reaction time: 24 hours



Conclusion

The Agilent Hi-Plex Ca column can be used to quantify the levels of the sugars in solution that result from the hydrolysis of cellulose.

A potentially useful application of this HPLC procedure is in the quality control of a glucose manufacture process or in the biofuels industry, where enzymes are often used to break down cellulose and hemicelluloses.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-01921

Agilent Technologies