

# Analysis of Byproducts in Fermentation Liquids Using an Agilent Hi-Plex H Column

## Application Note

Food and Beverage

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### Introduction

Biomass fermentation has grown in importance because diverse products such as fuel, lubricants, and chemicals can be derived. One option for this use of biomass is the fermentation of xylose from hemicelluloses, to xylitol, a sugar substitute. For the HPLC analysis of fermentation liquids, the US NREL Biomass Program method *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples* can be applied.



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## Materials and Methods

Two fermentation samples were analyzed. The first was obtained by a hydrothermal digestion of straw (as an example of biomass) that destroys the hemicelluloses and frees the xylose. Following partial evaporation of water, the second sample was obtained after fermentation of xylose to xylitol.

### Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 μm (p/n PL1170-6830)
Mobile phase	0.005 M H <sub>2</sub> SO <sub>4</sub>
Gradient	Isocratic
Flow rate	0.7 mL/min
Injection volume	20 μL
Sample concentration	Xylose ~ 8 g/L Glucose ~ 1.5 g/L Xylitol ~ 13 g/L Furfural 10 ~ 500 mg/L Hydroxymethylfurfural ~ 100 mg/L Acetic acid ~ 1000 mg/L Ethanol ~ 2000 mg/L Lactic acid ~ 2500 mg/L
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

## Results

After hydrothermal digestion, a large quantity of xylose is present in solution, as expected (Figure 1). Figure 2 shows that further fermentation of the sample converts a large quantity of this xylose into xylitol and gives a very large RI response for this sugar alcohol.

## Conclusion

The Agilent Hi-Plex H column is specially suited for the analysis of byproducts and degradation products (acids, alcohols, furfural, hydroxymethylfurfural), such as those produced by biomass fermentation. The column is recommended for use with samples that contain high levels of organic acids or for simultaneous analysis of these acids and sugars, using sulfuric acid as the mobile phase.

## For More Information

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Peak identification for Figures 1 and 2

- 1 Glucose
- 2 Xylose
- 3 Arabinose
- 4 Xylitol
- 5 Lactic acid
- 6 Glycerol
- 7 Acetic acid
- 8 Ethanol
- 9 Hydroxymethylfurfural (HMF)
- 10 Furfural

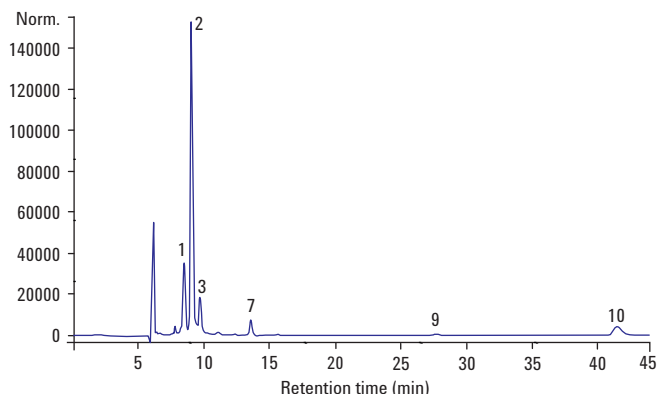


Figure 1. Analysis of a sample of straw after hydrothermal digestion using an Agilent Hi-Plex H column.

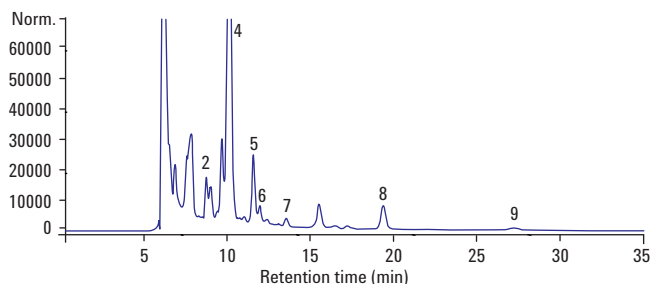


Figure 2. Components of a straw sample after fermentation of xylose to xylitol.

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© Agilent Technologies, Inc., 2011  
Published in USA, June 30, 2011  
SI-1942