

# Analysis of Bioethanol Fermentation Products Using an Agilent Hi-Plex H Column

## Application Note

Biofuels

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### Introduction

The fermentation of biomass to ethanol is an economically important process. For the control of the fermentation, HPLC is the method of choice because it can separate and detect carbohydrates (starting material), acids (potential inhibitors), fusel alcohols (byproducts), and ethanol (product) in one step without prior derivatization. This application note describes the analysis of a sample of a batch bioethanol fermentation product using an Agilent Hi-Plex H column.



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## Materials and Methods

The sample (a suspension of biomass and yeast in water) was pretreated by filtration through a 0.45 µm membrane.

### 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	Glucose ~ 37 g/L Xylose ~ 3.5 g/L Succinic acid ~ 1 g/L Lactic acid ~ 200 mg/L Glycerol ~ 10 g/L Acetic acid ~ 700 mg/L Methanol ~ 1.5 g/L Acetaldehyde ~ 300 mg/L Ethanol ~ 87 g/L
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

## Results

The chromatogram clearly indicates that the biomass sample contains large amounts of starting material, organic acids, byproducts, and final product mixed together.

## Conclusion

A sample from a batch fermentation was resolved with good separation using an Agilent Hi-Plex H column. Hi-Plex H columns are ideal for the analysis of sugar alcohols and sugar molecules using water as the mobile phase. Hi-Plex H is also the column of choice for the analysis of organic acids, using dilute acid as eluent. The use of a ligand-exchange chromatography column such as Hi-Plex H significantly reduces the need for complicated sample preparation (typically involving elution through an ion-exchange resin bed), as retention is brought about not only by ion exchange, but also by ion exclusion and partitioning on this type of column.

## For More Information

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### Peak identification

- 1 - 4 di- and oligosaccharides
- 5 Glucose
- 6 Xylose
- 7 Succinic acid
- 8 Lactic acid
- 9 Glycerol
- 10 Acetic acid
- 11 Acetaldehyde
- 12 Methanol
- 13 Ethanol

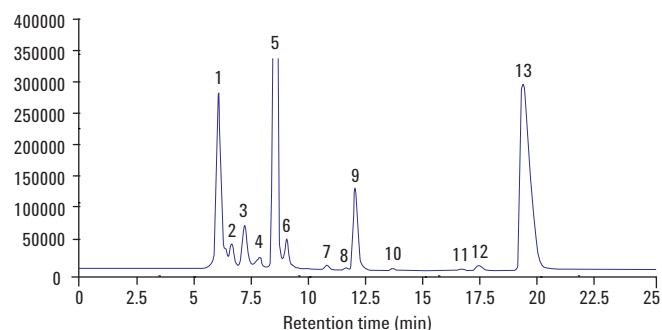


Figure 1. HPLC chromatogram of biomass and yeast in water, using an Agilent Hi-Plex H column.

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