Shodex™ HPLC approach for biomass related analysis

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Data provided by Showa Denko K.K.

Introduction:

Biomass is a renewable energy source obtained from biological materials. One type of useful product is biofuel. Biofuel is a general term used for the bioethanol and biodiesel. Bioethanol is the majority (~90%) of the biofuel used worldwide. It is considered as an ECO-friendly automobile fuel produced from sugar and starch in plants. Ideally the main source of biofuel is none-food source such as wood, algae, and inedible part of crops.

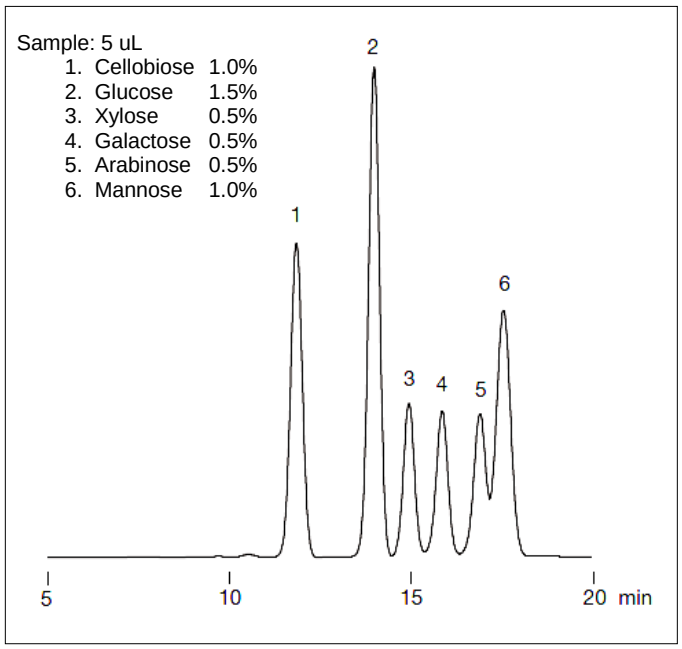
There are different biofuel production methods already used in industries, and the others that are under development. Most starting product is polysaccharide which needs to be broken down to monosaccharide in order to produce ethanol through fermentation process. Throughout the production, it is important to analyze various components generated. They are for quality assurance purpose of raw material, final product, byproduct, and impurities, as well as for understanding of degradation mechanism and for recovery monitoring of final product. HPLC analysis is the most frequently used qualification and quantification method. Several methods using Shodex™ columns are introduced in this article.

Contents:

Results:

1. Saccharides in wood

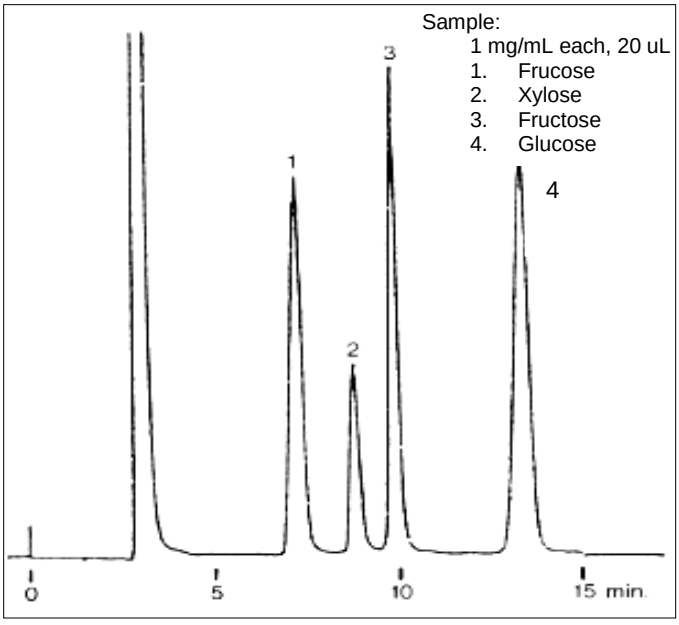
Polysaccharide hydrolyzation is a prerequisite for biomass production: As oligomers need to be further processed into monosaccharides in order to produce bioethanol. Separation of oligosaccharides and monosaccharides can be achieved under simple HPLC conditions using Shodex SUGAR SP0810 column. It works under size exclusion and ligand exchange modes. This method is used as a laboratory analytical procedure at National Renewable Energy Laboratory (http://www.nrel.gov/biomass/analytical_procedures.html: Technical Report NREL/TP-510-42618 Determination of Structural Carbohydrates and Lignin in Biomass 2008).



Column: Shodex SUGAR SP0810
Eluent: H_2O Flow Rate: 0.6 mL/min
Column Temperature: 85°C Detector: RI

2. Separation of hexose and pentose

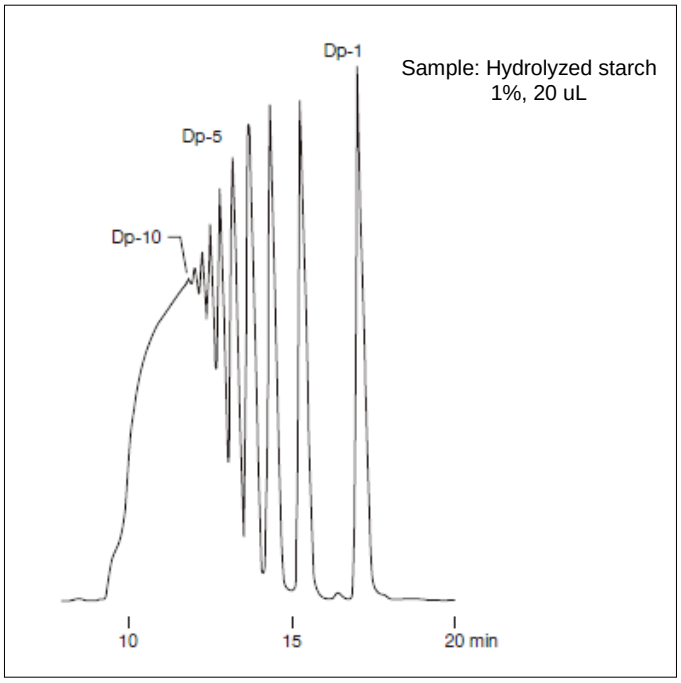
Both hexose and pentose are the source of bioethanol. However, there is less number of enzymes that can decompose pentose compared to the enzyme that can decompose hexose. Shodex Asahipak NH2P-50 4E, an amino HILIC column can be used to separate several hexose and pentose under isocratic elution.



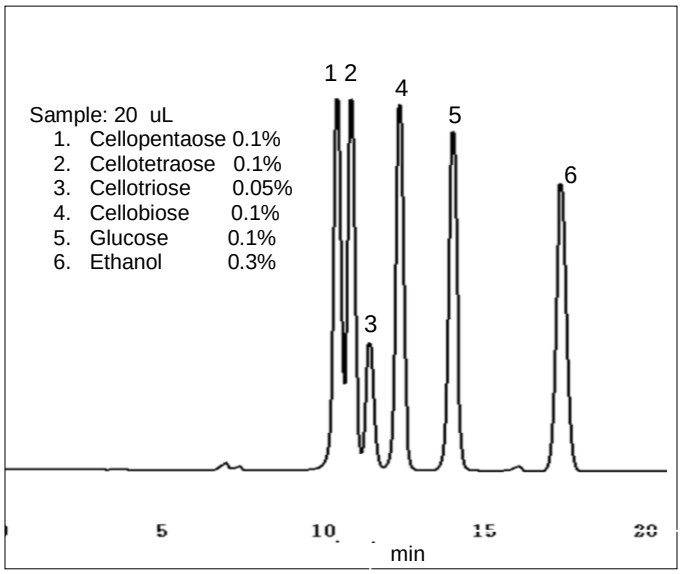
Column: Shodex Asahipak NH2P-50 4E
Eluent: CH_3CN/H_2O=80/20
Flow Rate: 1.0 mL/min
Column Temperature: 30°C Detector: RI

3. Hydrolyzed dextran I

Hydrolyzation process can be monitored by analyzing oligosaccharides with different molecular sizes. Shodex SUGAR KS series columns are suitable for the separation of polysaccharides under size exclusion mode. The same column can be also used to separate oligosaccharides from ethanol.



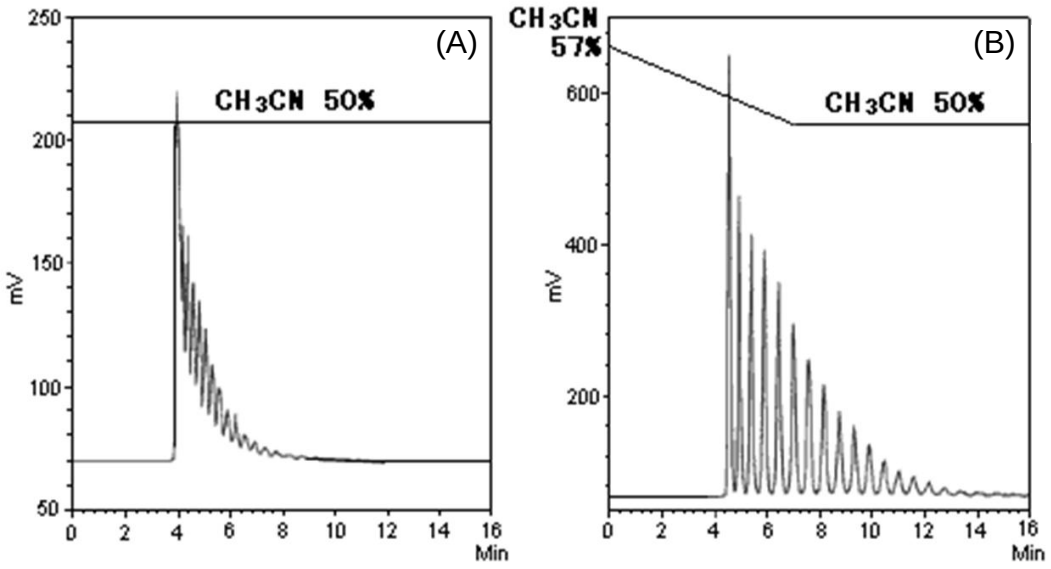
Column: Shodex SUGAR KS-802 x 2
Eluent: H_2O Flow Rate: 1.0 mL/min
Column Temperature: 80°C Detector: RI



Column: Shodex SUGAR KS-802
Eluent: H_2O Flow Rate: 0.6 mL/min
Column Temperature: 80°C Detector: RI

4. Hydrolyzed dextran II

Amino HILIC column is also suitable for hydrolyzation process monitoring. Gradient method provides better separation compared to an isocratic method. Evaporative light scattering detector (ELSD) is feasible for the detection of saccharides under gradient separation mode.

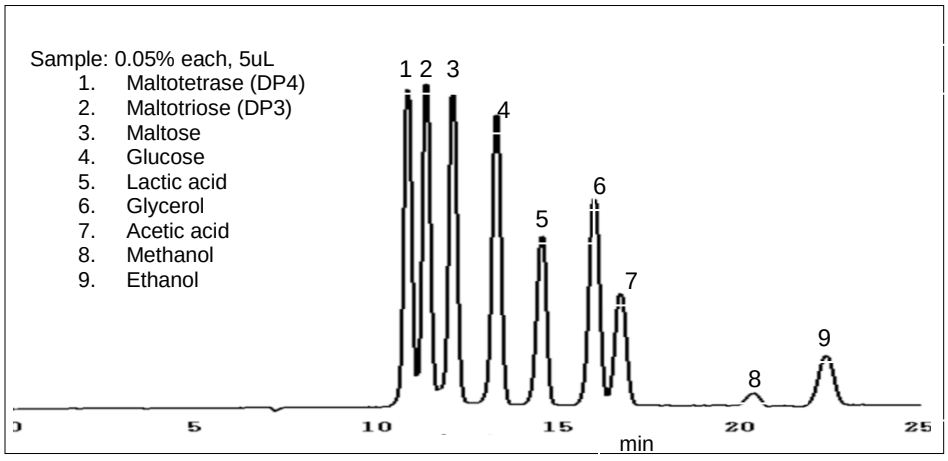


Sample: Hydrolyzed starch
0.5%, 20 uL

Column: Shodex Asahipak NH2P-50 4E
Eluent: (A) CH_3CN/H_2O=50/50
(B) 0 to 7min CH_3CN/H_2O=57/43 to 50/50 (Linear gradient), 7 to 16min CH_3CN/H_2O=50/50
Flow Rate: 1.0 mL/min Column Temperature: 30°C Detector: ELSD

5. Fermentation inhibitors (Organic acids)

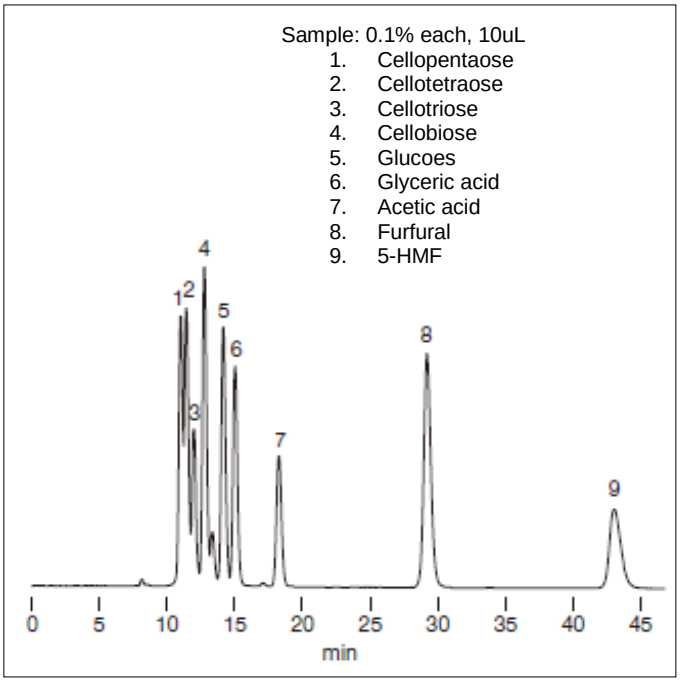
Organic acids are one of biomass hydrolyzation byproducts. They prevent microorganism (i.e., enzyme) growth, and thus inhibit fermentation process. Shodex SUGAR SH1821 column works under size exclusion and ion exclusion modes. It is suitable for the simultaneous separation of saccharides, organic acid, and alcohol. Elution time of organic acids can be controlled by optimizing the sulfuric acid concentration. For a better separation of DP4 and DP3, slower flow rate (0.6 mL/min instead of 1.5 mL/min) is recommended.



Column: Shodex SUGAR SH1821 Eluent: 0.5 mM H_2SO_4 (aq) Flow Rate: 0.6 mL/min
Column Temperature: 75°C Detector: RI

6. Fermentation inhibitors (Furfurals)

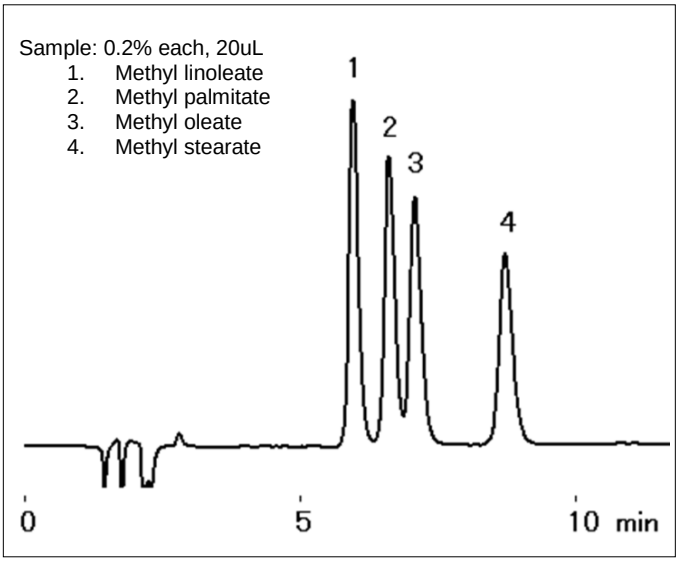
Depending on the type of biomass and processing mechanisms, furfural and 5-hydroxymethylfurfural (5-HMF) may be generated. They are considered as fermentation inhibitors. Shodex SUGAR SH1821 is suitable for the separation of celloligosaccharides and furfurals, using a combination of size exclusion and ion exclusion separation modes.



Column: Shodex SUGAR SH1821
Eluent: 2 mM H_2SO_4 (aq) Flow Rate: 0.6 mL/min
Column Temperature: 75°C Detector: RI

7. Biodiesel composition analysis

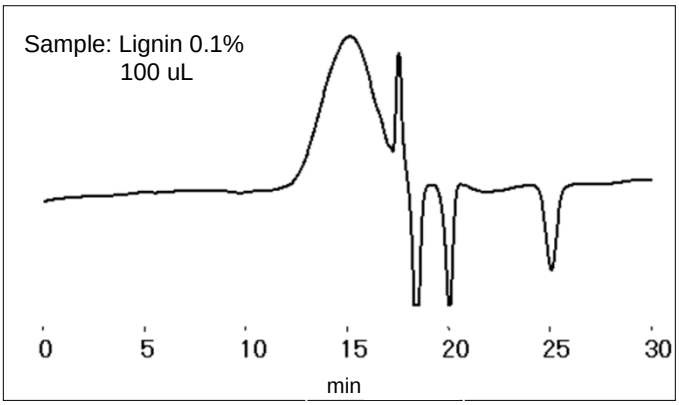
Fatty acid methyl esters are the components of biodiesel. They were analyzed using a polymer-based reversed-phase column; Shodex RSpak DS-413.



Column: Shodex RSpak DS-413
Eluent: $CH_3CN/THF/H_2O$=45/30/25
Flow Rate: 1.0 mL/min
Column Temperature: 40°C Detector: RI

8. Biomass (Lignin)

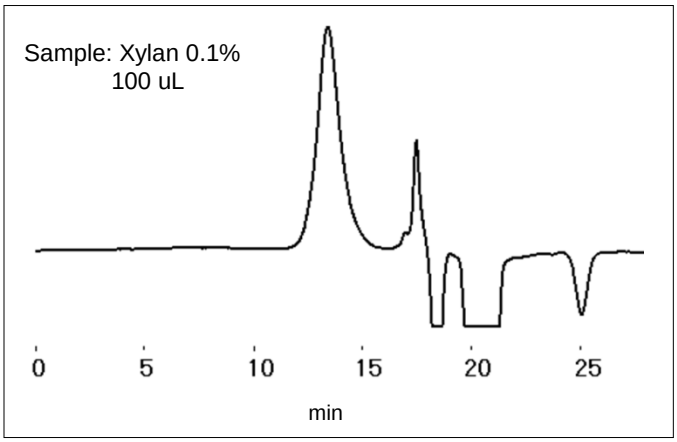
Lignin is present in the cell wall of wood and other plants. Although it is an undesired component for the production of biofuel, the degradation products of lignin have potential use in biopolymers. Shodex GPC LF-804 is a linear-calibration type GPC column, suitable for a wide range of MW determination analysis.



Column: Shodex GPC LF-804
Eluent: 20 mM H_3PO_4 + 20mM LiBr
in DMSO/DMF=80/20
Flow Rate: 0.6 mL/min
Column Temperature: 50°C Detector: RI

9. Biomass (Xylan)

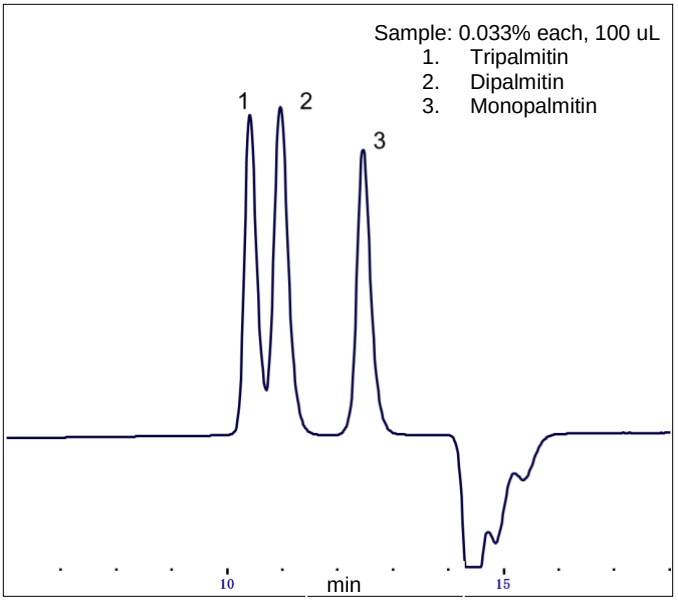
Xylan is a type of hemicellulose and present in most plants' cell wall. Hemicellulose, as well as cellulose, is used as a biofuel source. Hemicellulose is easily decomposed by weak acid or weak base and enzymes. Shodex GPC LF-804 is suitable for the MW determination and/or for xylan decomposition monitoring.



Column: Shodex GPC LF-804
Eluent: 20 mM H_3PO_4 + 20mM LiBr
in DMSO/DMF=80/20
Flow Rate: 0.6 mL/min
Column Temperature: 50°C Detector: RI

10. Impurities (Glycerides)

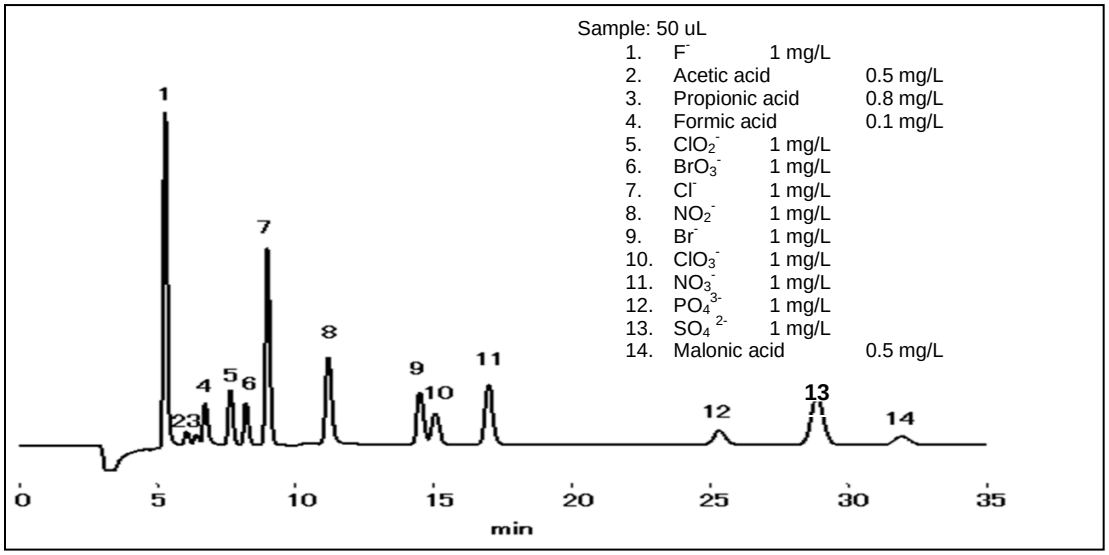
Glycerides are one of impurities found in biodiesel. Shodex Asahipak GF-310 HQ works under size exclusion mode to separate mono-, di- and tri-palmitins.



Column: Shodex Asahipak GF-310 HQ
Eluent: THF Flow Rate: 0.6 mL/min
Column Temperature: 30°C Detector: RI

11. Impurities (Organic acids)

Acetic, propionic, and formic acids are impurities of biofuel. They may cause fuel tank corrosion. Shodex IC SI-52 4E is a strong-anion chromatography column. It provides simultaneous separation of organic acids, oxyhalides, and other anions.



Column: Shodex IC SI-52 4E Eluent: 3.6mM Na_2CO_3 (aq)
Flow Rate: 0.8 mL/min Column Temperature: 45°C Detector: Suppressed conductivity

Conclusions:

Shodex™ HPLC columns offer a number of separation modes to analyze various components generated during biomass production. Presented methods include the analysis of raw material, final products, byproducts, impurities, as well as methods for saccharification/hydrolization monitoring.

Concentration and composition of mobile phase, temperature, and/or flow rate influence the separation performance of particular HPLC column. Therefore, for the specific set of target analysis, optimization of HPLC condition is highly recommended.

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