LC/UV/MS analysis of monitoring bioethanol manufacturing process with using polymer-based multi-solvent SEC column

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Abstract

The production of bioethanol begins with pyrolysis of bagasse, enzymatic conversion into saccharides, and finally fermentation. In order to improve productivity, saccharides as well as inhibitors (e.g. organic acids and furfurals) of fermentation should be measured.

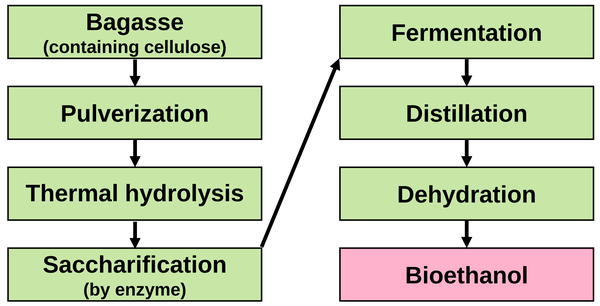
Typically, saccharides are analyzed by ligand exchange chromatography, while organic acids and furfurals are analyzed separately by ion exclusion/reversed-phase chromatography. In order to simplify the analysis, a simultaneous LC/UV/MS method was studied.

A multi-solvent size exclusion chromatography (SEC) column with poly vinyl alcohol base packing material, Shodex Asahipak GF-210 HQ was used. A mixed standard solution containing glucose, cellooligosaccharides (2-5 saccharide units) and various organic acids was analyzed. The saccharides eluted from the column in accordance with SEC mode, followed by elution of organic acids and furfurals. Retention of the organic acids was attributed to hydrophobic interaction with the packing material rather than SEC mode. Furfurals were detected more sensitively by UV than by MS.

A sample obtained by pyrolysis of bagasse and enzymatic conversion to saccharides was also analyzed. The analysis showed the standard substances as well as xylooligosaccharides, organic acids originated from hemicellulose, and benzaldehydes originated from lignin.

Our method using simultaneous LC/UV/MS is effective for analysis of bioethanol production.

Manufacturing process of bioethanol



- The components (organic acids, furfurals, and so on) produced in thermal hydrolysis interfere with fermentation, so these products should be analyzed in order to control them.
- Saccharides and these products have been ordinarily measured at different conditions (SEC or ion exclusion/ reversed-phase chromatography).

Simultaneous analysis of all compounds in one condition is very efficient .

Aqueous and organic SEC columns Asahipak GF-HQ series

Product specifications and line up

Product name	Exclusion Limit (Pullulan)	Plate Number (TP/Column)	Particle Size (μm)	Column Size I.D. × Length (mm)
GF-210 HQ	9,000	≥ 19,000	5	7.5 × 300
GF-310 HQ	40,000	≥ 19,000	5	7.5 × 300
GF-510 HQ	300,000	≥ 19,000	5	7.5 × 300
GF-710 HQ	(10,000,000)*	≥ 11,000	9	7.5 × 300
GF-7M HQ	(10,000,000)*	≥ 13,000	9	7.5 × 300
GF-1G 7B	-	(guard column)	9	7.5 × 50

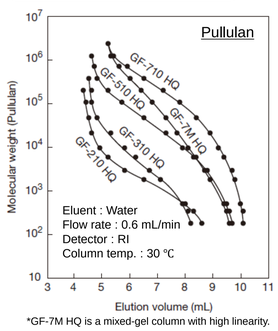
( )* Estimated value

Packing material : Poly vinyl alcohol particle
Housing : Stainless steel
Usable temp. : 4～60 ℃ (recommend 25～40 ℃)
Usable pH range : 2～9
Max flow rate : 1.0 mL/min (recommend 0.4～0.6 mL/min)
Max pressure : 7 MPa (GF-210 HQ and GF-310 HQ)
Usable solvents : Water, Methanol, Ethanol, Acetonitrile, THF Acetone, DMF, Chloroform, and DMSO (0~50%)

Polymer based

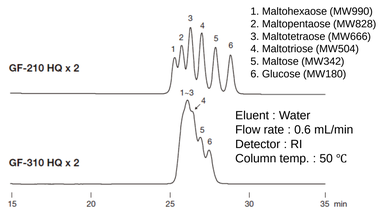
Aqueous and various organic solvents available

Calibration curves

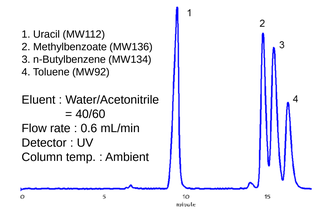


*GF-7M HQ is a mixed-gel column with high linearity.

Separation of maltooligosaccharides



Utilizing of hydrophobic interaction

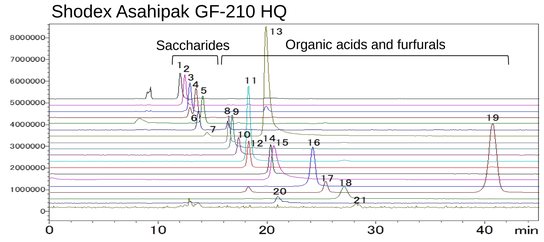


- GF-210 HQ is suitable for the separation of oligosaccharides than GF-310 HQ.
- Hydrophobic interaction with the packing material is available for the separation.

【Result 1】 Simultaneous analysis of the mixed standard

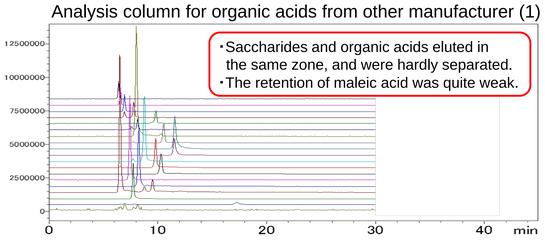
Mass chromatograms of the mixed standard

Shodex Asahipak GF-210 HQ



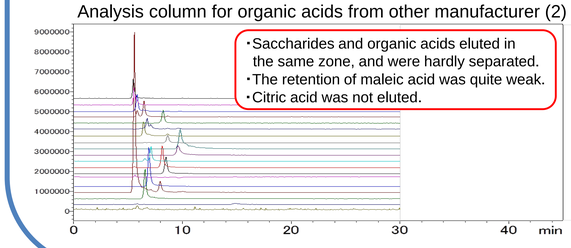
Sample : 5 μg/mL each（in water）, 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : shown above each data
Eluent : (A) Water/Formic acid = 100/0.1 (v/v)
(B) Acetonitrile
Isocratic ; (B%) 20%
Flow rate : 0.6 mL/min
Column temp. : 50 ℃
Detector : PDA & ESI-MS (SIM)

Analysis column for organic acids from other manufacturer (1)



- Saccharides and organic acids eluted in the same zone, and were hardly separated.
- The retention of maleic acid was quite weak.

Analysis column for organic acids from other manufacturer (2)

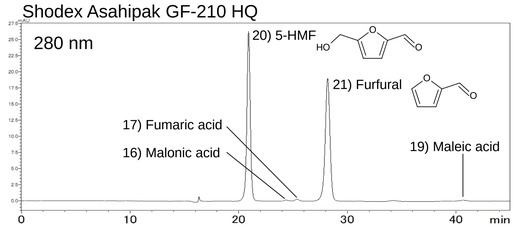


- Saccharides and organic acids eluted in the same zone, and were hardly separated.
- The retention of maleic acid was quite weak.
- Citric acid was not eluted.

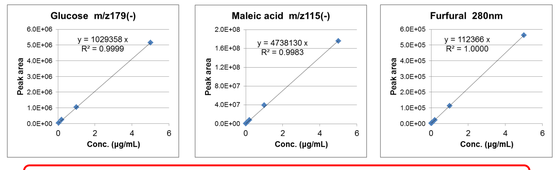
Peak No.	Compound	Monitored m/z
1	Cellopentaose	827(-)
2	Cellotetraose	665(-)
3	Cellotriose	503(-)
4	Cellobiose	341(-)
5	Glucose	179(-)
6	Xylobiose	281(-)
7	Xylose	149(-)
8	Glyceric acid	105(-)
9	Glycolic acid	75(-)
10	Lactic acid	89(-)
11	Malic acid	133(-)
12	Succinic acid	117(-)
13	Tartaric acid	149(-)
14	Adipic acid	145(-)
15	Citric acid	191(-)
16	Malonic acid	103(-)
17	Fumaric acid	115(-)
18	Pyruvic acid	87(-)
19	Maleic acid	115(-)
20	5-HMF	127(+)
21	Furfural	97(+)

UV chromatogram of the mixed standard

Shodex Asahipak GF-210 HQ



Calibration curves

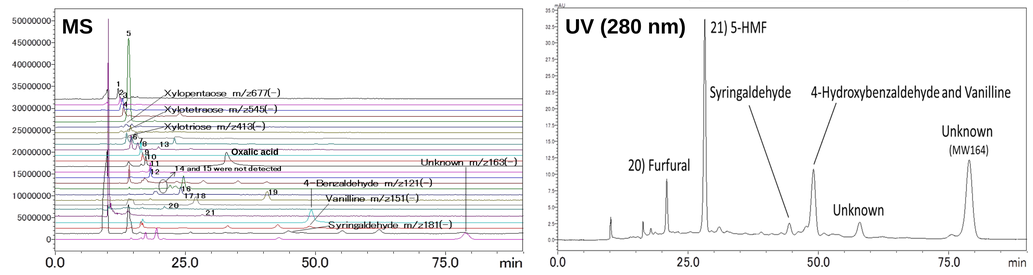


High linearity & high sensitivity (0.05 μg/mL detectable-each)

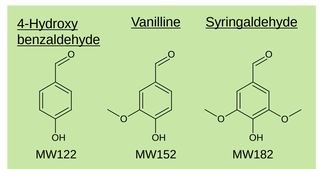
- Using Asahipak GF-210 HQ, saccharides were separated by SEC mode, and organic acids and furfurals were separated by reverse-phase mode after the elution of saccharides.
  But saccharides and organic acids were eluted in the same zone, using two analysis columns for organic acids from other manufacturers.
- Furfurals were sensitively detected by UV.
- LC/UV/MS using GF-210 HQ is very useful for simultaneously analyzing saccharides and other compounds such as organic acids and furfurals.

【Result 2】 Analysis of the actual sample

Chromatograms of the saccharification sample



- Three kinds of benzaldehydes known as inhibiters of fermentation derived from lignin were detected by UV and MS.
- The main two unknown peak were detected at 58min and 79min on UV Chromatogram. These components may be novel benzaldehydes.
- This method was applicable for analysis of the thermal hydrolysis sample and saccharification sample (The data of the thermal hydrolysis sample is not shown here).
- Analysis time is estimated to become shorter by increasing the ratio of acetonitrile.



Conclusion

LC/UV/MS method with GF-210 HQ enables simultaneously analyzing saccharides, organic acids, furfurals, and benzaldehydes.

This method provides an efficient and sensitive analysis of the samples of bioethanol manufacturing process.