SHIMADZU
Excellence in Science

Application News

No.L485

High Performance Liquid Chromatography

Analysis of Biomass Using Organic Acid Analysis System

Total worldwide energy consumption is steadily growing year-by-year, particularly as a result of economic development and population growth in emerging countries. As a result, there is increasing demand for fossil fuels, including oil and coal, natural gas, etc., while there is growing concern about the depletion of these energy sources. In addition, concern about the environmental impact of energy consumption is also intensifying, all of which are serving to focus attention on the development of alternative fuels.

Biomass is a carbon neutral resource that is widely available on the earth, and is naturally regenerated. Effective use of timber from forest thinning and agricultural waste has advantages, both environmentally and economically. Industrial production of bioethanol from these unused biomass resources involves a process in which cellulose and hemicellulose in the biomass are decomposed using diastatic enzymes to be to converted to monosaccharides such as glucose or xylose, which are then converted to ethanol by the action of yeast.

Efficient decomposition of the biomass using diastatic enzymes requires pretreatment with an acid or alkali. However, as a result of this pretreatment, organic acids such as formic acid and acetic acid, and furan compounds such as furfural and hydroxymethylfurfural are produced as byproducts, which adversely inhibit fermentation by the yeast. Here, we introduce an example of simultaneous analysis of acetic acid, formic acid, furfural, and 5-hydroxymethyl furfural, typically present in the biomass, using a combination of an organic acid analysis system together with an ultraviolet-visible spectrophotometric detector.

Analysis of Standard Solution

Fig. 1 shows a flow diagram of the system that was used for this analysis. In an organic acid analysis system, pH buffer solution is mixed with the column eluate to bring its pH to near-neutral, thereby promoting dissociation of the organic acids and improving sensitivity for their detection in the electrical conductivity detector. Here, formic acid and acetic acid were detected by the electrical conductivity detector in the organic acid analysis system. However, as furfural and 5-hydroxymethylfurfural are difficult to detect using an electrical conductivity detector, they were detected by the UV-VIS detector downstream of the electrical conductivity detector.

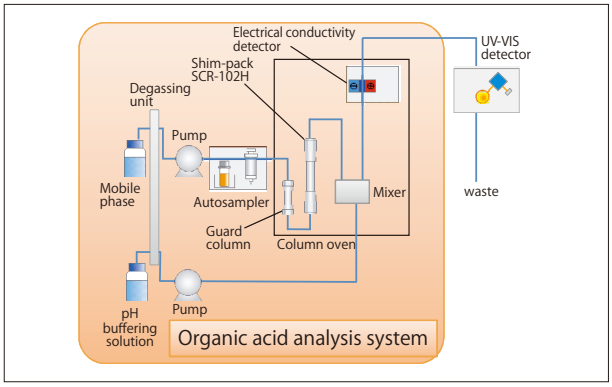

Fig. 1 System Flow Diagram

Table 1 shows the analytical conditions. To permit the combined use of the UV-VIS detector, a perchloric acid aqueous solution, which exhibits little UV absorption, was used as the mobile phase. Fig. 2 shows the results obtained using a 10 µL injection of a standard solution of 4 substances typically present in biomass (each at 10 mg/L).

Table 1 Analytical Conditions

Column	: Shim-pack SCR-102H (300 mm L, 8.0 mm I.D.)
Guard Column	: Guard column SCR-102H (50 mm L, 6.0 mm I.D.)
Mobile Phase	: 5 mmol/L perchloric acid aq. solution
Flowrate of Mobile Phase	: 0.8 mL/min
pH Buffering Solution	: 5 mmol/L perchloric acid containing 20 mmol/L Bis-Tris and 0.1 mmol/L EDTA
Flowrate of pH Buffering Solution	: 0.8 mL/min
Column Temp.	: 35 °C
Detection 1	: Electrical conductivity detector (CDD-10Avp) (Formic acid, acetic acid)
Detection 2	: UV-VIS detector (SPD-20AV) at 275 nm (5-hydroxymethylfurfural, furfural)
Injection Vol.	: 10 µL

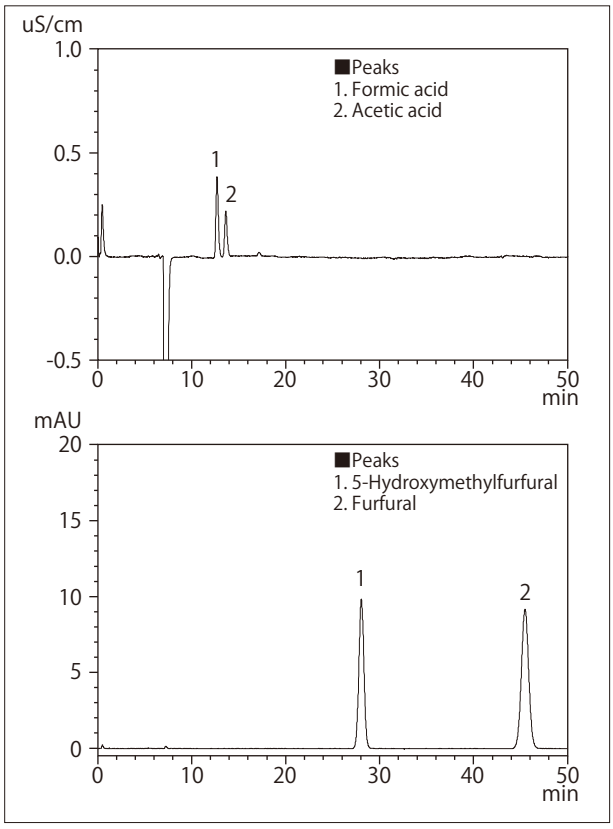

Fig. 2 Chromatograms of Standard Mixture (10 mg/L each) Upper: Electrical Conductivity Detector, Lower: UV-VIS Detector

Linearity of Calibration Curves

Fig. 3 shows the calibration curves based on analyses using the analytical conditions of Table 1. The calibration curves were generated over the concentration ranges 10 to 400 mg/L for formic acid and acetic acid, and 1 to 400 mg/L for furfural and 5-hydroxymethylfurfural. Excellent linearity was obtained for each substance, with coefficients of determination greater than "$R^2 = 0.9999$" in all cases.

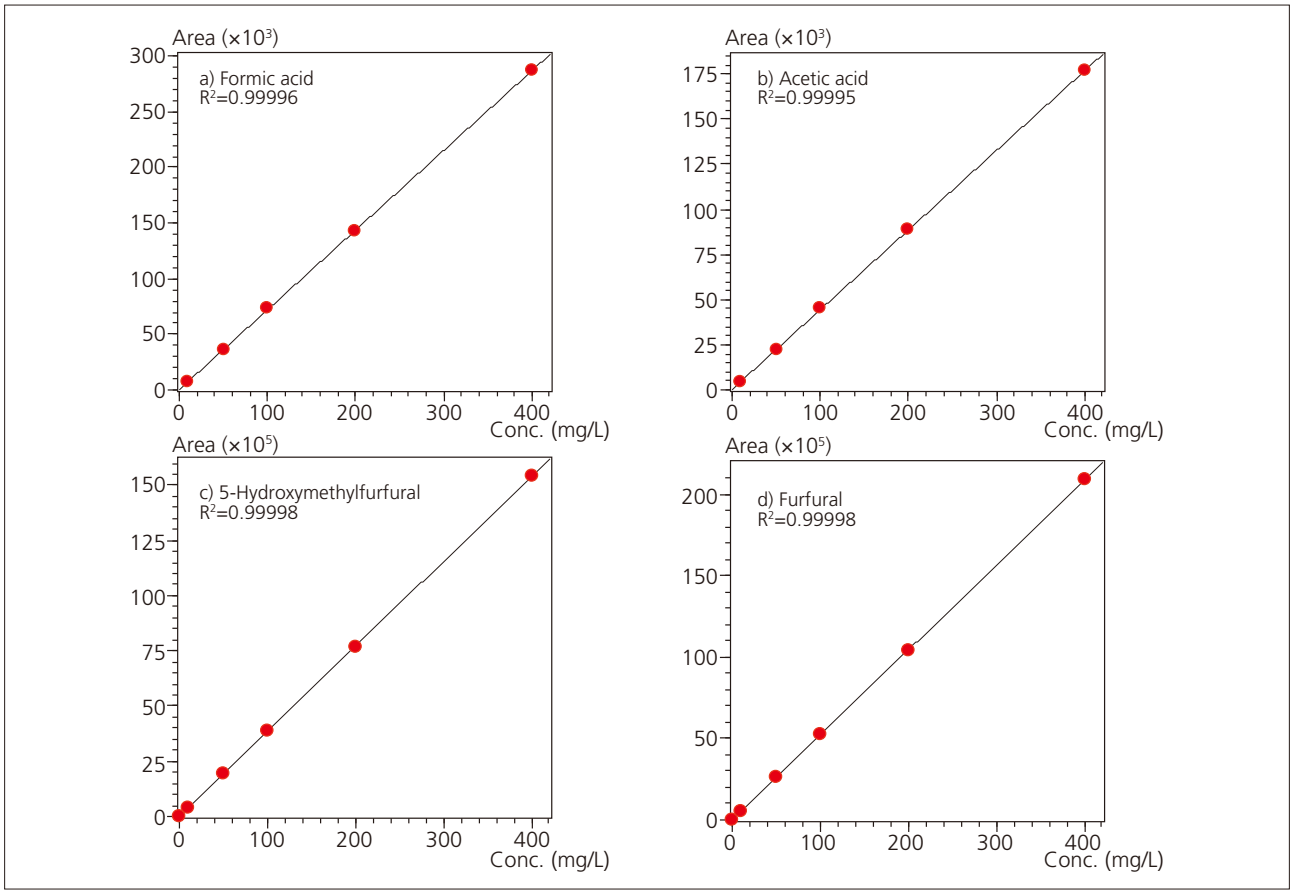

Fig. 3 Linearity of Calibration Curves
a) Formic Acid, b) Acetic Acid, c) 5-Hydroxymethyl furfural, d) Furfural

Analysis of Biomass

Fig. 4 shows the result of analysis of the filtrate obtained following hydrothermal processing of plant-derived biomass, and Table 1 shows the analytical conditions. By combining the electrical conductivity detector with the UV-VIS detector, good separation was obtained between the biomass components and impurities contained in the biomass.

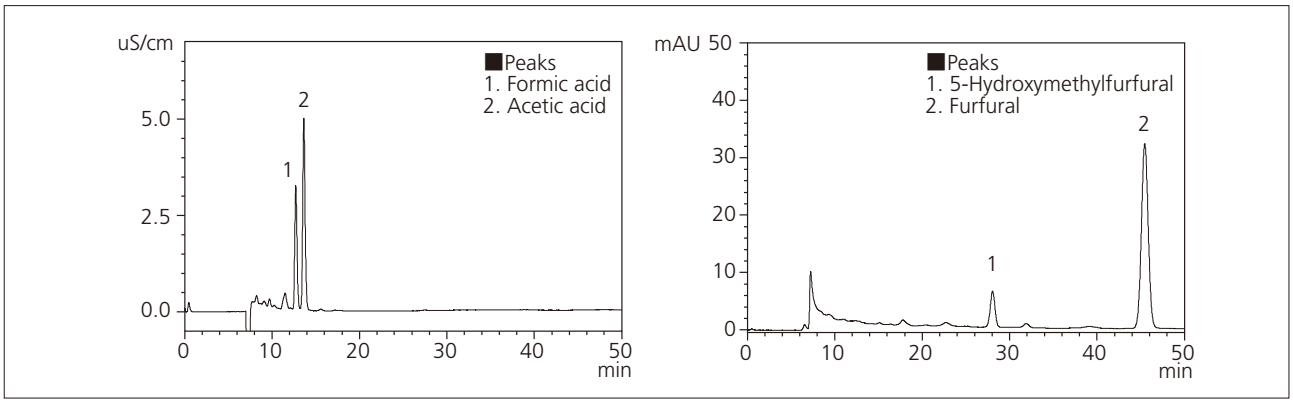

Fig. 4 Chromatograms of Biomass
Left Side: Electrical Conductivity Detector, Right Side: UV-VIS Detector

First Edition: Feb. 2015

Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.
© Shimadzu Corporation, 2015