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Rapid determination of formic and acetic acids in biomass hydrolysate by headspace gas chromatography

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ABSTRACT

A headspace gas chromatography (HS-GC) method is developed for determination of formic acid (FA) and acetic acid (AA) in biomass hydrolysate. The method is based on the "in-vial" derivatization reaction of alcohols and acids. $NaHSO_4$/ethanol is selected as the preferred derivatization reagent. The method has a reproducibility of RSD <0.87% and recovery of 97.1%–103.1%. The limits of the quantification were 2.02 and 2.38 mg/L for FA and AA, respectively. The GC analysis time only required 12.4 min. The method is rapid, sensitive and suitable for measuring FA and AA in the multifarious biomass hydrolysates in pulping and biorefinery industries.

Introduction

With the reduction of fossil energy resource which is used as a dominate energy and chemical source to sustain our present way of life, the renewable forest material has been attracted considerable attention recently [1]. The integrated forestry bio-refinery (IFBR) is a concept for utilizing biomass materials high efficiently and therefore providing additional environmental and economic benefit [2]. Hemicelluloses are the most easily and valuably separable main component of biomass materials and can be converted to high valued-added products [1,3]. Pre-hydrolysis (mainly hot water, weak acid and alkali extractions) is an important step to extract hemicelluloses in the processes of biomass materials and product generation [4–6]. During hydrolysis pretreatment, the acetic acid (AA) is generated by the cleavage of acetyl groups in the hemicelluloses [7]. And under the acidic conditions, hemicelluloses are hydrolyzed to oligosaccharide and monosaccharide, and partially further decomposed into 5-(hydroxymethyl) furfural (HMF) and furfural subsequently. And then the HMF and furfural both can degrade to formic acid (FA) [8,9]. The contents of formic and acetic acids play a crucial role in the extraction of hemicelluloses, the degradation of cellulose and the dissolution of lignin [10,11]. Those acids can be as catalysts for oligosaccharides dissolution during the pretreatment and may lead to degradation of carbohydrate [11]. Hydrolysis pretreatment prior to high-yield pulping often leads to some reduction of pulping yield and some of the resultant pulp properties due to the changes of carbohydrates content and pulp fiber's structure, which are closely related to the formic and acetic acids released from biomass materials [10]. Therefore, it is of great significance to quantify formic and acetic acids in the biomass hydrolysate.

Several analytical techniques have been applied for quantification of formic and acetic acids, including traditional titration methods [12], high performance liquid chromatography (HPLC) [13], ion chromatography (IC) [14] and gas chromatography (GC) [15] and etc. The traditional titration methods are commonly not selective, and the results are variable and generally showed as the content of total acids [12,16,17]. For the IC methods, it is necessary to clean up the samples with extensive and cumbersome pretreatment [18,19]. Numerous people have tended to choose HPLC with FID detection for the analysis of formic and acetic acids. But it is time-consuming, relatively low sensitivity and for the sample matrix of biomass hydrolysate. And several researchers also commonly used HPLC with ultraviolet (UV) detection to determine the formic and acetic acids [20]. But due to the lack of any significant chromophore for organic acids, high molar absorptions occurred only at short UV wavelengths, which could compromise the method selectivity.

Compared with these methods mentioned above, headspace gas chromatograph (HS-GC) technique has some unique advantages [21] due to the inherent advantages of GC (e.g., simplicity, high sensitivity, and short running time) and HS sampler (e.g., automated and solvent-free). HS-GC techniques can largely eliminate above-mentioned flaws and provide relatively automatic and solvent-free analyses [22]. However, HS-GC has been also faced with some critical problems while determining formic and

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acetic acids, such as poor accuracy and high detection limits, due to the hydrophilic, high polar, and semi-volatile characteristics of formic and acetic acids [23]. Therefore a derivatization step is necessary prior to the HS-GC analysis [24]. Although there are some well-established derivatization methods for fatty acids [24,25], for the special matrix of biomass hydrolysate, it has never been reported in the published literatures. In addition, the derivatization step was often carried out in a water bath outside HS-GC for a long time, and then for the further analysis [26]. This derivatization approach not only led to a long pretreatment time, but also reduced the precision of the HS-GC analysis.

In this paper, we report a recommendable method that couples convenient "in-vial" derivatization with HS-GC to measure formic and acetic acids in the biomass hydrolysate. Prior to GC analysis, the simultaneous derivatization/volatilization of formic and acetic in a HS-vial was directly performed in the oven of the HS sampler instead of outside derivatization. In this way, during the derivatization reaction, the sample was balancing at the same time which can save plenty of time and the most errors caused by manual sampling also can be minimized. To get a higher selectivity and sensitivity, the performance of seven derivatization reagents were selected and compared, and factors such as the dosage of $NaHSO_4$, the conditions of sample equilibration and chromatographic separation were optimized and validated. And the difference between the present HS-GC and the HPLC methods was also explored.

Materials and methods

Chemicals

FA (>98.00% purity) and AA (>99.99% purity) were purchased from Sigma-Aldrich (Switzerland). Ultrapure water was prepared by a Mili-Q Plus 185 water purification system (Milipore, USA). All other chemicals used in this study were of analytical grade unless otherwise indicated. The stock solutions with a concentration of 16.00 g/L were prepared by diluting the reagents of formic and acetic acids with ultrapure water, respectively, and then stored in a refrigerator at 4 °C. The standard solutions of formic and acetic acids were prepared by diluting the stock solutions with ultrapure water before experiment.

Materials

The hydrolysates were obtained from the autohydrolysis process of poplar chips in a 6 L digester (M/K systems Inc., USA) by using the following conditions: an equivalent of 300 g (oven-dried) chips, a liquid/solid ratio of 8:1 (kg/kg), an autohydrolysis temperature of 160 °C for 60 min. After the pretreatments, the hydrolysates were acidified using 10% sulfuric acid to a pH value of 2.5. Then they were centrifuged at 4500 rpm for 15 min to separate the insoluble compounds. In order to evaluate the application of the present HS-GC method for different kinds of test samples, various hydrolysate samples, including the hydrolysates from acid hydrolysis of poplar chips, autohydrolysis of wheat straw, and alkaline hydrolysis of eucalyptus chips, were collected from different labs.

"In-vial" derivatization procedure

Prior to GC analysis, an "in-vial" derivatization of formic and acetic acids was conducted [27]. The derivatization reagent refers to esterifying agent coupled with catalyst. The optimization of derivatization reagents and conditions were explored, and the detailed concentrations and dosages are listed in Table 1. First, the derivatization reagent was placed into a 20 mL HS-vial. Then a 2.5 mL of the sample and a 0.5 mL of methanol or ethanol were added. Subsequently, the vial was sealed immediately. The sealed vial was then placed in the HS sampler for derivatization reaction and subsequent determination of formic and acetic acids by HS-GC automatically.

Table 1
Type, concentration and dosage of the derivatization reagents. Q5

Derivatization reagent	Concentration	Dosage
$NaHSO_4 + CH_3OH$	3.5 g + 0.5 mL[a]	
$NaHSO_4 + CH_3CH_2OH$		
$BF_3 + CH_3OH$	10%[b]	1.5 mL
$HCl + CH_3OH$	1:3 (v/v)[c]	1.5 mL
$HCl + CH_3CH_2OH$	1:3 (v/v)	1.5 mL
$H_2SO_4 + CH_3OH$	1:3 (v/v)	1.5 mL
$H_2SO_4 + CH_3CH_2OH$	1:3 (v/v)	1.5 mL

[a] The dosage of $NaHSO_4$ and methanol or ethanol are 3.5 g and 0.5 mL, respectively.
[b] The concentration of BF_3 in CH_3OH is 10%.
[c] The volume ratio of the catalyst to alcohol is 1:3.

HS-GC working conditions

HS sampling was conducted using a model G1888 network HS sampler from Agilent Technologies (USA). The HS operating conditions were listed as follows: shaking time of 30 min at 80 °C, vial pressurization time of 0.20 min, sample loop fill time of 0.15 min, loop equilibration time of 0.05 min, and injection time of 0.50 min.

GC analysis was performed with an Agilent 7890A GC system from Agilent Technologies (USA) with a flame ionization detector (FID). Chromatographic separations were carried out using an HP-5 fused silica capillary column (30 m × 0.32 mm ID × 0.25 μm film thickness) and an HP-INNOWax silica capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness) both from Agilent J&W Advanced Capillary GC Columns (USA). The temperatures of injection and detection were both 260 °C and the injection was operated at a 5:1 split mode. Nitrogen with high purity (≥99.99%) was used as carrier gas and kept constant flow rate at 1 mL/min. The GC oven initial temperature was 60 °C and held for 0.5 min, then programmed to rise to 100 °C at 5 °C/min and continually to 200 °C from 100 °C at 30 °C/min. The whole GC analysis process required only 12.4 min.

HPLC analysis

The HPLC method was according to the US National Renewable Energy Laboratory (NREL) [28]. The HPLC analysis was performed with an Agilent Technologies 1200 Series system (USA) with refractive index detection using a Biorad Aminex HPX-87H column (300 mm × 7.8 mm). The mobile phase was 5 mmol/L H_2SO_4 and with a flow rate of 0.6 mL/min at a temperature of 55 °C. A 10 μL of test sample was injected to the HPLC system and the complete sample elution was accomplished within 50 min with the detector temperature of 55 °C.

Method linearity, precision, detection limit and recovery

Linearity was evaluated in terms of the R^2 value of the linear regression equations. Calibration lines were obtained with twelve concentration levels of the standard solutions within the range of 0.005–16.00 g/L, and each level was measured in triplicate.

Precision was investigated by calculation of repeatability and reproducibility. For repeatability, it was determined by analysis of the standard solution of formic and acetic acids at five different concentration levels of 0.05, 0.1, 0.2, 0.5 and 1.0 g/L. Each concentration was measured in triplicate. The reproducibility of

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