Journal of Chromatography A, 1218 (2011) 5319–5327

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Direct and simultaneous determination of representative byproducts in a lignocellulosic hydrolysate of corn stover via gas chromatography–mass spectrometry with a Deans switch

Rongping Zheng [a], Hongman Zhang [a,*], Jing Zhao [a], Mingliu Lei [a], He Huang [b]

[a] College of Science, Nanjing University of Technology, Nanjing 210009, China
[b] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 9 March 2011
Received in revised form 26 May 2011
Accepted 30 May 2011
Available online 12 June 2011

Keywords:
GC–MS
Deans switch
Byproduct
Lignocellulosic hydrolysate

ABSTRACT

Pretreatment is one of the most important steps in producing fuel ethanol from lignocellulosic biomass. Simple, fast and accurate quantification of byproducts in lignocellulosic hydrolysates is critical to optimize the pretreatment procedures, but still a challenge. In this paper, a new GC–MS (SIM) method based on a Deans switch has been developed for the determination of byproducts in a corn stover hydrolysate. The Deans switch was incorporated into a hardware system that facilitated the direct aqueous injection (DAI) on GC–MS system. Simultaneous chromatographic separation and quantification of 18 byproducts including four aliphatic acids, five furan derivatives, four phenolic compounds and five others were achieved within 45 min. The detection limits of the presented method for various byproducts were in the range of 0.007–0.832 mg/L. The within-day and between-day precisions of the method were less than 6.0% (RSD, $n = 6$). The accuracy of the method was confirmed with recoveries of 86–128%. A lignocellulosic hydrolysate sample of corn stover was successfully analyzed using this method, with aliphatic acids and furan derivatives accounting for 89.15% of the selected total byproducts.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Producing fuel ethanol from lignocellulosic biomass has several benefits including domestic availability, pollution reduction and ease of limited fossil fuel resources, and has been a research focus around the world. Generally, the lignocellulosic biomass is compact structure of cellulose (30–40%), hemicellulose (15–30%) in close association with lignin (15–30%) [1,2]. The biomass can be corn stover, wheat straw, bagasses, and wood chips, but cannot be directly utilized for the production of ethanol without a pretreatment which aims at disrupting the lignocellulose matrix and rendering cellulose more accessible for enzymatic digestion. The common pretreatment methods include milling, thermal treatment, acid treatment, alkaline treatment, and treatment with organic solvents or a combination of these methods [3,4]. In industry, the mostly used methods are dilute acid hydrolysis [1], compressed-hot water hydrolysis [2], and steam explosion [5].

However, those hydrolysis procedures based on the treatment of lignocellulose at high temperature and acidic condition would inevitably lead to the formation of a range of inhibitory byproducts. Generally, the byproducts are divided into three major categories based on their origins: aliphatic acids, furan derivatives and phenolic compounds [6]. These compounds are major barriers for the conversion of biomass to ethanol due to the interference with microbial fermentation of sugars [7]. To reduce the negative effect of these byproducts on the following fermentation, hydrolysate must be treated with various detoxification methods [8–10]. The detoxification process also could be avoided by reducing the byproduct formation through optimization of the pretreatment conditions (hydrolysis temperature, time, and acid concentration) [6,11]. Therefore, quick and accurate identification and quantification of the byproducts are desired to optimize pretreatments and overcome the problems associated with them.

The byproducts are so complicated that their analysis could not be achieved with a single analysis technology. The quantification of the three main groups was normally performed using several technologies. For the determination of aliphatic acids, gas chromatography (GC) [12–15], high performance liquid chromatography (HPLC) [16–18] and capillary electrophoresis (CE) [19] have been employed. Most of the analytical protocols for furan derivatives are based on HPLC techniques with UV detection but suffer from the separation of target ana-

* Corresponding author. Tel.: +86 25 83172094; fax: +86 25 83172094.
E-mail addresses: hmzhang9607@sina.com, hmzhang@njut.edu.cn (H. Zhang).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.05.099

lytes from the complex biological matrix [20]. There are many publications investigating the phenolic compounds in plants [21–25]. The GC and GC–MS systems, in particular, enable the separation and identification of lignin degradation products. However, these methods have the disadvantage of requiring time-consuming steps: extraction, concentration, and derivatization.

Tremendous efforts have been made towards the identification and quantification of degradation byproducts in lignocellulosic hydrolysates [26–30]. Chen et al. report a reversed-phase HPLC technique with UV detection and an excellent extraction procedure has been applied for the determination of aliphatic acids, aromatic acids and neutral degradation products [26]. National renewable energy laboratory (NREL) utilizes HPLC with refractive index detection for simultaneous quantification of byproducts (not including phenolic compounds) in the dilute acid hydrolysate of corn stover [27]. Recently, liquid chromatography–tandem mass spectrometry (LC–MS/MS) and flow injection electrospray mass spectrometry (FIE-MS) have also been developed to qualify and quantify multiple byproducts (nearly 40 compounds) in lignocellulosic hydrolysates [28,29]. In the most recent research, Humpula et al. determine the major byproducts such as acetic acid, furfural, and acetamide by GC–MS with a DB-Wax polar column [30]. However, most of these relevant methods also have complicated sample pretreatment [26,28], require very expensive and advanced equipment (such as triple quadrupole mass spectrometer) [28,29], or focus on the quantification of a limited number of byproducts [27,30].

Generally, the extraction and enrichment of the analytes by complicated sample pretreatment in HPLC or GC methods may cause errors in the determination of volatile compounds such as formic acid, acetic acid, and furfural, and also lead to the loss of trace compounds such as phenol and guaiacol. Mass spectrometry (MS) has been broadly applied to both the qualitative and quantitative analyses because of its identification capability of unknown inhibiting byproducts. For the detection of aquatic samples with MS, however, there are some limitations due to the damage of water to the non-polar column and the mass source.

In this paper, a direct aqueous injection (DAI) without complicated sample pretreatment was carried out by GC–MS equipped with a DB-FFAP polar column and a micro-fluidic Deans switch. The Deans switches have been used in the heart-cutting of comprehensive two-dimensional gas chromatography for more than two decades [31–35]. The objectives of this study were: (1) to employ the new DAI GC–MS method to identify byproducts in the lignocellulosic hydrolysate of corn stover and (2) to develop a sensitive and accurate GC–MS method with selected ion monitoring mode (SIM) for the simultaneous determination of aliphatic acids, furan derivatives and phenolic compounds.

2. Experimental

2.1. Reagents and samples

Standards of hydroxyacetone, 2,6-dimethoxy phenol, 3-hydroxy pyridine, 5-methyl furfural, 5-hydroxymethyl furfural (5-HMF), 2-furoic acid as well as pyrrole-2-carboxaldehyde were purchased from Sigma–Aldrich (USA). Formic acid, acetic acid, propionic acid, levulinic acid, 3-hydroxy-2-butanone, furfural, furfuryl alcohol, 3-methyl-1,2-cyclopentanedione, guaiacol, phenol, vanillin and isopentanol (used as internal standard) were obtained from Aladdin-reagent Co., Ltd., Shanghai, China. All standards were prepared as stock solutions in methanol (HPLC grade). A series of solutions with internal standard were prepared for the construction of calibration curves. The concentration of the internal standard in every working solution and analyzed sample was 0.30 g/L.

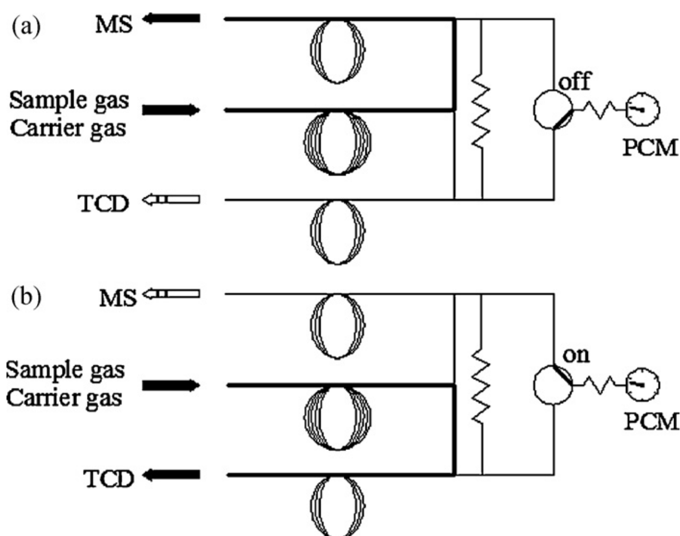

Fig. 1. Schematic flow diagrams of DAI GC–MS system: (a) Deans switch valve in "off" position; (b) Deans switch valve in "on" position.

2.2. Preparation of hydrolysate

Chips (80-mesh < size < 20-mesh) of corn stover (Lianyungang, China) were impregnated with 40 mL 1.0 wt% sulfuric acid per 4 g solid. The hydrolysis was performed in a self-made batch reactor (stainless steel, 11.1 cm × 3.3 cm id, 45 mL). The reactor was put into an oil bath (HH-S, Jiangsu, China) with an initial temperature of 30 °C and a heating rate of 5 °C/min. The biomass material was treated at 180 °C for 30 min after heating-up. The reaction product was discharged into a collection vessel, and the solid/liquid fractions were then separated by 100% polyester cloth. The hydrolysate had a pH of 2.0–2.5. After the hydrolysate (6.0 mL) was conditioned with NaOH to a pH of 4.5 and added with internal standard solution, it was diluted to 10.0 mL. Then the sample was filtrated with a super membrane syringe filter (0.22 μm pore size) and subjected directly to the split/splitless inlet.

2.3. DAI GC–MS system

The DAI GC–MS system consists of a split/splitless inlet, GC column, host GC oven, Deans switch, thermal conductivity detector (TCD), and MS. The outlet of the GC column was connected to the middle metal ferrule on Deans switch plate. Inlets of the same two restrictors were connected to the other two metal ferrules. One outlet was directed to TCD, and the other to MS. The hardware details of GC system equipped with the Deans switch are listed in Table 1.

Fig. 1 shows the schematic flow diagrams of DAI GC–MS system. The sample was injected into the split/splitless inlet and separated on the DB-FFAP column. The sample gas from the column could be fully diverted to either MS detection or TCD by the Deans switch. The swing of sample gas between the two detectors was controlled by a pneumatics control module (PCM). Whichever detector got "blocked" by auxiliary flow would be compensated by the auxiliary flow as new carrier gas, and the effluent from the column would be totally diverted to the other detector.

With the solenoid valve in "off" position, the DB-FFAP effluent was directed to MS (Fig. 1a). Just before the methanol peak eluted from the GC column, the valve was automatically set to "on" position and the column effluent was redirected to TCD (Fig. 1b). After methanol and water have been cut to TCD, the valve would be set back to "off" position. Thereby, the Deans switch was designed as a water "vent" to avoid its damage to the mass source and facilitate the DAI on GC–MS system. Lignocellulosic hydrolysates can be

Table 1
GC configuration equipped with a Deans switch.

Agilent 7890A GC	
Inlet	Split/splitless inlet with EPC control
GC column	DB-FFAP capillary column (30 m × 0.25 mm id, 0.25 μm film)
GC column connections	In: Split/splitless inlet; out: Deans switch
Restrictor 1	1.0 m × 0.10 mm id deactivated silica tubing
Restrictor 1 connections	In: Deans switch; out: TCD
Restrictor 2	1.0 m × 0.10 mm id deactivated silica tubing
Restrictor 2 connections	In: Deans switch; out: MS
Pressure control	Agilent 7890A Pneumatics control module (PCM)
Detector	Thermal conductivity detector, TCD
Deans switch	Micro-fluidic Deans switch kit (Agilent part no. G2855B) including Deans switch calculator software

Table 2
Deans switch conditions.

Micro-fluidic Deans switch	
Injection port EPC pressure	39.217 psi helium, constant pressure mode
DB-FFAP column flow	1.5 mL/min, constant pressure mode
Pneumatics control module (PCM)	31.687 psi helium, constant pressure mode
Cut time start	4.10 min
Cut time end	7.00 min

directly injected to be separated on the GC column and detected on MS.

Cut times for water (RT 6.51 min) and methanol (RT 4.35 min) were determined by injecting a pure water or methanol sample with the solenoid valve set in "on" position during the entire run. All cut times were automatically controlled through the Agilent 7890A timed event table. Electronic pneumatics control pressures, flow rates, and the fixed restrictor dimensions were determined using a Deans switch calculator. This calculator program is included with the Deans switch hardware option for Agilent 7890A GC. Relative details about the Deans switch conditions are shown in Table 2.

2.4. GC–MS instrumental analysis

Byproducts in the lignocellulosic hydrolysate were analyzed on Agilent 5975C MSD/7890A GC. The GC was equipped with a DB-FFAP column. Optimized operating conditions were as follows: injector temperature, 250 °C; injection mode, split at a ratio of 10:1; injection volume, 1 μL; oven temperature program, the initial temperature maintained at 70 °C, heated to 140 °C at 5 °C/min, heated to 180 °C at 8 °C/min and held for 10 min, finally heated to 230 °C at 5 °C/min and held for 6 min; TCD detector temperature, 220 °C; transferline temperature, 250 °C; mass source temperature, 230 °C (Electron Impact mode, EI: 70 eV); quadrupole mass analyzer temperature, 150 °C; carrier gas, helium with a flow of 1.5 mL/min; solvent delay, 3 min. The full-scan (a mass range of 30–500 amu) and SIM modes were employed for qualitative and quantitative analyses, respectively. In addition, the optimization of the instrumental conditions to improve resolution of analytes in Section 3.3 was carried out with the GC–MS operating in full-scan mode.

3. Results and discussion

3.1. Functionality of Deans switch

The full results of MS and TCD equipped with the Deans switch in analyzing a hydrolysate sample are shown in Fig. 2. It was found that the injection of water to MS could be effectively cut by the switch. Because the detection of many analytes does not require any derivatization or complicated sample pretreatment prior to GC–MS analysis, the prospects for the application of the Deans switch in DAI GC–MS method are good.

As shown in Fig. 2, formic acid eluted after acetic acid, which was confirmed by retention times of standards. This disagreed with the order of their boiling points. The same result was also obtained by Hong et al. with detailed explanation [36]. The lower pK_a value of formic acid and more water in contact with the column stationary phase in DAI mode enhanced the hydrogen bonding, which played a more important role in determining the elution order of the two acids than the boiling points.

3.2. Confirmation of unknown byproducts

Identification of byproducts was performed by MS equipped with EI source (70 eV) and quadrupole mass analyser operating in full scan mode. The structures of unknown compounds were initially matched with the mass spectra NIST library. Then the results should be evaluated by the following mass spectral fragmentation analysis: confirming the base peak, matching the abundance ratio of fragment ions, revealing the assignment of key ions, etc. In addition, the structure properties of lignocellulosic material and characterization of byproducts from the relevant literatures should also be consulted. As listed in Table 3, 20 byproducts were found in the dilute acid hydrolysate of corn stover, including four aliphatic acids, five furan derivatives, five phenolic compounds and six others. Most of the matching probability values returned by the NIST library were higher than 70.

18 byproducts were further confirmed by retention times of standards. 2,3-Dyhydro benzofuran and 2-methoxy-4-vinyl phenol were not able to be quantified because no standards were available.

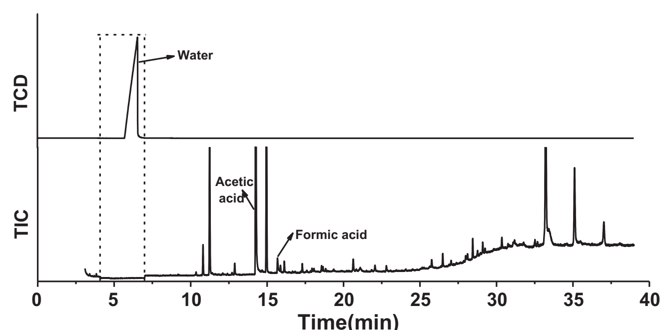

Fig. 2. Full results of MS and TCD in analyzing a hydrolysate sample.

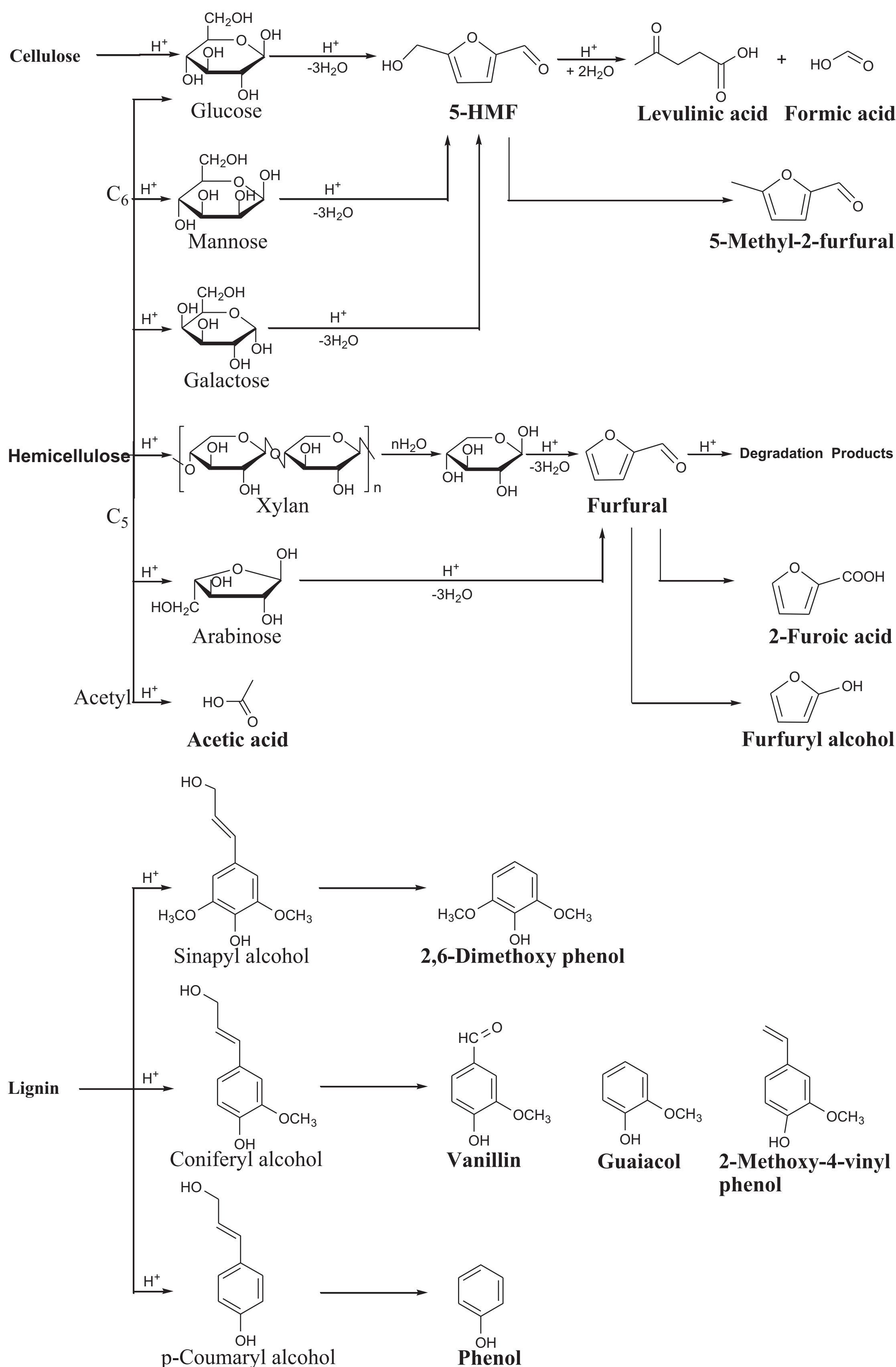

Fig. 3. The formation paths of representative byproducts in lignocellulosic hydrolysates.

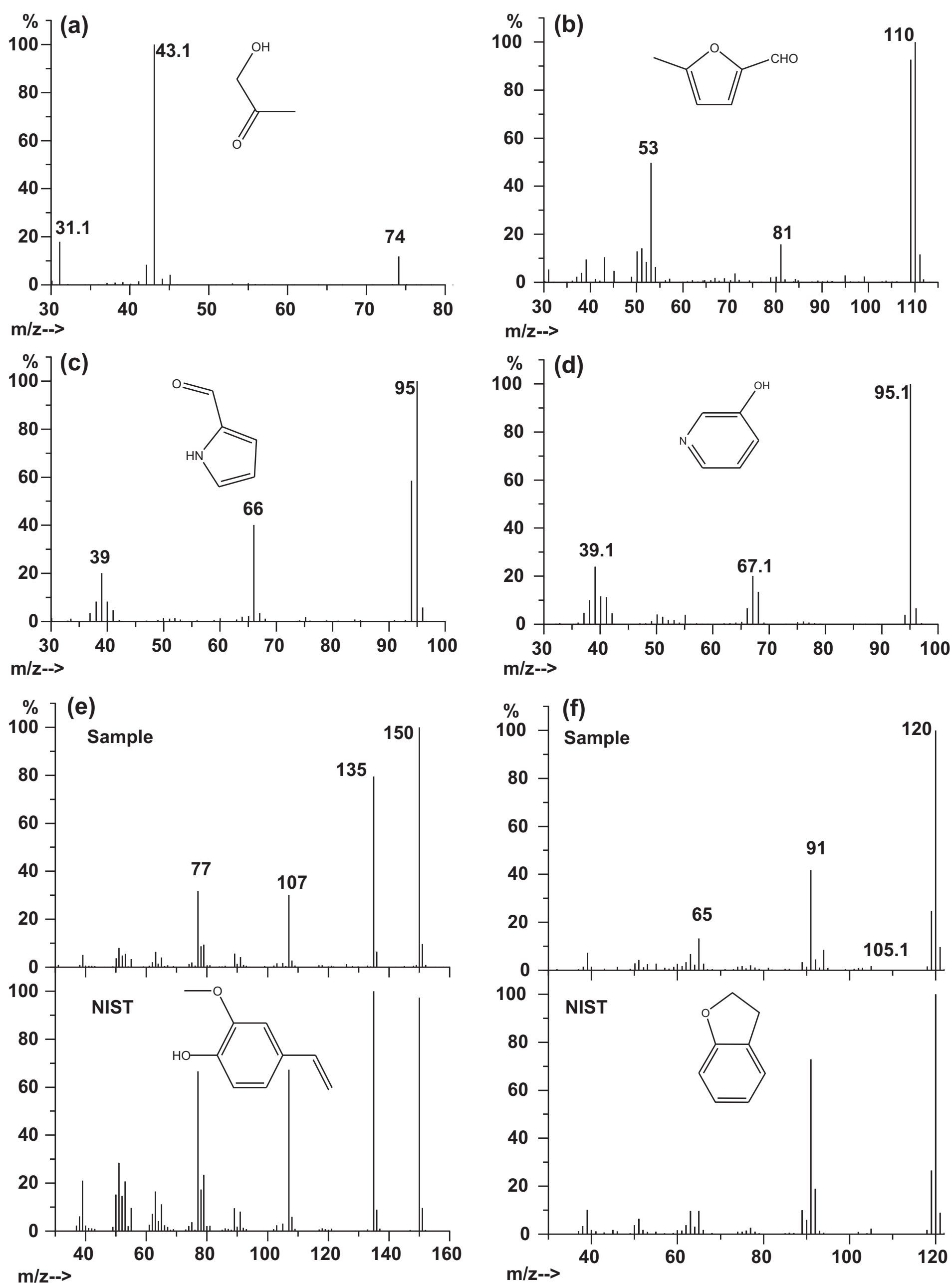

Fig. 4. Chemical structures of several selected analytes and their corresponding spectrums: (a) hydroxyacetone; (b) 5-methyl furfural; (c) pyrrole-2-carboxaldehyde; (d) 3-hydroxy pyridine; (e) 2-methoxy-4-vinyl phenol; (f) 2,3-dyhydro benzofuran.

The formation paths of representative inhibitory byproducts are summarized in Fig. 3 [6,9,11,37,38]. Acetic acid is mainly derived from acetyl groups in hemicellulose. Degradation of monosaccharide in the pretreatment and hydrolysis steps leads to the formation of furfural and 5-HMF, which may further degrade to aliphatic acids, such as formic acid and levulinic acid. A wide range of phenolic compounds with different substituents are formed during the degradation of lignin. The origin of 3-hydroxypyridine could also be found in the structure of lignocellulose [39].

Several less-reported byproducts and their mass spectra extracted from the chromatograms of hydrolysate sample are illustrated in Fig. 4. The NIST library suggested the unknown compounds to be: (a) hydroxyacetone, MW = 74; (b) 5-methyl furfural, MW = 110; (c) pyrrole-2-carboxaldehyde, MW = 95; (d) 3-hydroxy pyridine, MW = 95; (e) 2-methoxy-4-vinyl phenol, MW = 150; (f) 2,3-dyhydro benzofuran, MW = 120. Mass spectrum of compound a had a strong base peak at m/z 43 corresponding to characteristic fragment of acetyl group of hydroxyacetone (Fig. 4a). For compounds b and c, which were matched to be aromatic aldehydes, their base peaks seemed to coincide with the molecular ions (Fig. 4b and c). As aromatic aldehydes, there always is a prominent ion at $[M-1]^+$. The molecular ion of aromatic aldehyde with no substituent (such as pyrrole-2-carboxaldehyde) has a tendency to fragment by losing the aldehyde group, resulting in a relative strong ion at $[M-29]^+$. Compound d was indicated to be 3-hydroxy pyridine with the probability value of 87. The rearrangement of the hydrogen in the hydroxyl group to aromatic ring can cause ring-opening reaction upon the α-bond breakdown. Then $[M-CO]^{+\bullet}$ or $[M-HCO]^+$ fragment is produced, such as ions at *m*/*z* 68 and 67 for compound d in Fig. 4d.

The possible structures and the mass spectra (extracted from the chromatograms of hydrolysate sample and NIST library) of the

Table 3
Byproducts found in hydrolysate sample and the matching probability values.

Compound	Probability	Compound	Probability
Acetic acid	91	Phenol	90
Formic acid	72	2,6-Dimethoxy phenol	46
Propionic acid	80	2-Methoxy-4-vinyl phenol[a]	91
Levulinic acid	68	Vanillin	74
Furfural	95	Hydroxyacetone	72
5-Methyl-2-furfural	93	3-Hydroxy-2-butanone	80
Furfuryl alcohol	89	3-Methyl-1,2-cyclopentanedione	74
2-Furoic acid	87	2,3-Dyhydro benzofuran[a]	72
5-HMF	86	Pyrrole-2-carboxaldehyde	93
Guaiacol	90	3-Hydroxypyridine	87

[a] Not confirmed and quantified with standards.

two compounds without confirmation with standards are shown in Fig. 4e and f. Compounds e and f exhibited fragment ions at m/z 77, 66, 65, 51, 40 and 39 indicating that they contained phenyl function group. For compound e, the probability of being 2-methoxy-4-vinyl phenol was as high as 91 according to the NIST library. Moreover, the structure of 2-methoxy-4-vinyl phenol coincides with the monolithic structure of lignin [40]. For compound f, the coexistence of fragment ions at 91 and 105 showed the presence of a methylene group and the strong fragment ion at m/z 91 indicated the existence of benzyl group. NIST library suggested compound f to be 2,3-dyhydro benzofuran with a probability of 72. We also found that this structure unit in the structure of lignocellulose [41].

3.3. Optimization of the instrumental conditions

To achieve an adequate resolution of all analytes, many factors such as temperature and heating rate of the oven ramp, the final oven temperature and split ratio were studied. It was firstly necessary to optimize the oven temperature program to improve the separation of 3-hydroxypyridine and 2-furoic acid because they co-eluted as a peak under the original conditions of GC and their ionization via EI resulted in common ions. Ions at m/z 39 and 95 were the key fragmentation ions of 3-hydroxypyridine, and ions at m/z 39, 95, and 112 for 2-furoic acid.

The original oven temperature program for sample analysis was set as follows: (1) the initial temperature maintained at 70 °C; (2) heated to 140 °C at 5 °C/min; (3) heated to 180 °C at 8 °C/min and held for 10 min; (4) finally heated to 230 °C at 10 °C/min and held for 10 min. Tests showed that the final temperature of the third ramp and heating rate of the fourth ramp influenced the separation of 3-hydroxypyridine and 2-furoic acid greatly. Three different final temperatures of the third ramp (160 °C, 180 °C, and 200 °C) were studied. The peaks of 3-hydroxypyridine and 2-furoic acid overlaid mostly when the heating rate of the fourth ramp was 10 °C/min (Fig. 5b). Results showed that the worst final temperature was 160 °C. The separation of the two compounds under three different oven temperature programs is presented in Fig. 5. An increase of resolution was apparent as the final heating rate decreased to 5 °C/min and the final temperature of the third ramp was chosen at 180 °C rather than 200 °C (Fig. 5a and c).

To obtain peaks of all analytes and reduce the loss of stationary phase of GC column simultaneously, the final temperature of the oven temperature program was optimized at 230 °C. The boiling point of vanillin is 284 °C, which is highest among the analytes. Tests showed that vanillin could not be eluted from the column when the final temperature was 220 °C. However, final temperatures higher than 230 °C were not employed due to the loss of stationary phase. The maximum temperature that is recommended

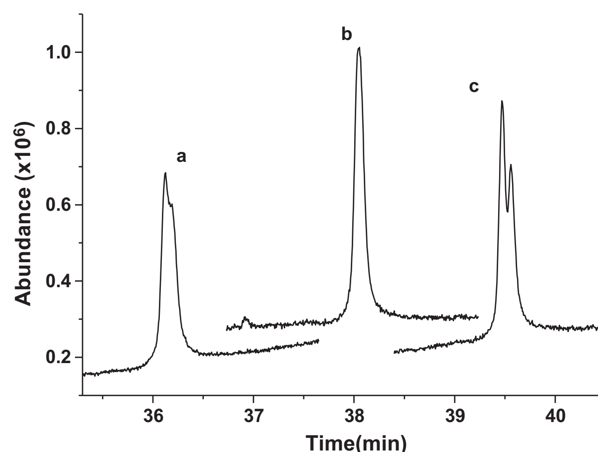

Fig. 5. The separation of 3-hydroxypyridine and 2-furoic acid under three different oven temperature programs. The final temperature of the third ramp and the heating rate of the fourth ramp were: (a) 200 °C, 5 °C/min; (b) 180 °C, 10 °C/min; (c) 180 °C, 5 °C/min.

by the manufacturer (J&W Scientific) for DB-FFAP-type open-tube columns is 250 °C.

Split ratios (50:1, 40:1, 30:1, 20:1, 10:1, splitless) were optimized to obtain a better sensitivity of analysis. It was found that some peaks were too broad and some with peak tailing at the splitless injection mode. For the sake of better protection of the column and analytical reliability, 10:1 was chosen for this method.

3.4. Method validation

3.4.1. Calibration curves, and limits of detection (LODs)

The concentration of analytes and relative peak areas to internal standard were used for the construction of calibration plot and quantification. All quantitative analyses were performed in SIM mode. Complete SIM parameters and retention times of the analytes are shown in Table 4. Four separate time windows were used for ions in SIM mode. Qualifier ions in Table 4 were the key fragment ions which were used to characterize the analytes. The extracted ion chromatogram of GC–MS (SIM) for a standard mixture is shown in Fig. 6. Most of the 18 analytes were separated well except 3-hydroxypyridine and 2-furoic acid.

A summary of the analytical data obtained is presented in Table 5. Good linearity was obtained for the developed GC–MS (SIM) method with correlation coefficients in the range of 0.9850–0.9994. Limits of detection (LODs) were calculated as the

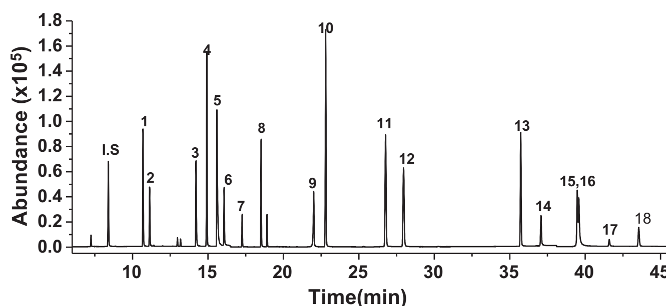

Fig. 6. The extracted ion chromatogram of GC–MS (SIM) for a standard mixture at the concentration of 0.1 g/L except for formic acid 1.0 g/L. Peak assignment: (1) 3-hydroxy-2-butanone; (2) hydroxyacetone; (3) acetic acid; (4) furfural; (5) formic acid; (6) propionic acid; (7) 5-methyl furfural; (8) furfuryl alcohol; (9) 3-methyl-1,2-cyclopentanedione; (10) guaiacol; (11) phenol; (12) pyrrole-2-carboxaldehyde; (13) 2,6-dimethoxy phenol; (14) levulinic acid; (15) 3-hydroxy pyridine; (16) 2-furoic acid; (17) 5-hydroxymethyl furfural; (18) vanillin; IS, internal standard.

Table 4
SIM parameters of GC/MS run.

Analyte	Retention time (min)	Start time (min)	Group (SIM ions)	Qualifier ions (m/z)	MW
Isopentanol	8.64	3	1 (13)	**55**[a], 70	88
3-Hydroxy-2-butanone	10.69			**45**[a], 88	88
Hydroxyacetone	11.13			**43**[a], 74	74
Acetic acid	14.21			**43**[a], 60	60
Furfural	14.93			39, 67, 95, **96**[a]	96
Formic acid	15.59			45, **46**[a]	46
Propionic acid	16.06			45, 73, **74**[a]	74
5-Methyl-2- furfural	17.26	16.50	2 (10)	53, 109, **110**[a]	110
Furfuryl alcohol	18.52			81, 97, **98**[a]	98
3-Methyl-1,2-cyclopentanedione	21.99			83, 84, **112**[a]	112
Guaiacol	22.79			81, **109**[a], 124	124
Phenol	26.75	26.00	3 (10)	65, 66, **94**[a]	94
Pyrrole-2-carboxaldehyde	27.95			66, 94, **95**[a]	95
2,6-Dimethoxyphenol	35.72			111, 139, **154**[a]	154
Levulinic acid	37.06			**43**[a], 56, 116	116
3-Hydroxypyridine	39.47	38.10	4 (8)	39, **95**[a]	95
2-Furoic acid	39.57			39, 95, **112**[a]	112
5-Hydroxymethylfurfural	41.60			**97**[a], 109, 126	126
Vanillin	43.54			109, 151, **152**[a]	152

[a] Qualifier ion in boldface type represents the most abundant ion of the compound.

average amount of analyte giving a response that is three times the noise (S/N = 3). As listed in Table 5, the method enabled the detection of the analytes in the range of 0.007–0.832 mg/L with acids possessing the lowest sensitivity. This provided adequate detection limits of analytes.

3.4.2. Method precision and repeatability

To verify the precision of the proposed method, within-day and between-day precision of the standard mixture at the concentration level of 0.1 g/L except for formic acid 1.0 g/L were evaluated. The relative standard deviations (RSDs, $n = 6$) of within-day and between-day repeatability of retention times were within 0.1% and 0.9%, respectively. The within-day and between-day RSDs ($n = 6$) of peak area repeatability were all within 6.0% except 2-furoic acid and 5-HMF which were not very stable. The between-day precisions of peak area for 2-furoic acid and 5-HMF were 7.17% and 9.44%, respectively (Table 6).

The precision was further assessed by replicate analyses of the dilute acid hydrolysate of corn stover (every replicate was injected twice). The three replicate analyses of the hydrolysate sample showed RSDs (0.2–1.2%) for the retention times of selected analytes (data not shown). RSDs for the relative peak areas to internal standard of the different compounds ranged between 3.64 and 12.73% (Table 7). This demonstrated good repeatability of sample preparation as well as the stability of the DAI GC–MS system.

3.4.3. Recovery and matrix effects

The accuracy of the developed method was assessed by the standard addition method. In this way, the influence of the matrix on method reliability was ascertained. The method of standard addition was performed by analyzing unspiked lignocellulosic hydrolysate and spiked hydrolysate. The spiked hydrolysate was prepared from the unspiked hydrolysate by adding a standard mixture with the amount of each analyte 0.5, 1 or 2 times as much as the unspiked sample. As listed in Table 7, the obtained recoveries of the 18 analytes ranged between 86 and 128%. This demonstrated adequate accuracy of the method.

3.4.4. Sample analysis

Practicability and applicability of the method were confirmed by the determination of the 18 analytes in a lignocellulosic hydrolysate of corn stover. The hydrolysate prepared at 180 °C for 30 min with 1.0 wt% sulfuric acid was analyzed in triplicate. The results of analysis are in Table 7. The contents of aliphatic acids and furan derivatives were calculated to account for 50.60% and 38.55% of the total selected byproducts in the corn stover hydrolysate, respectively.

Table 5
Linear regression on the GC–MS (SIM) response versus concentration for analytes and LOD.

Analyte	Calibration curve	Linear range (mg/L)	R^2 ($n = 6$)	LOD (mg/L)
3-Hydroxy-2-butanone	$y = 3.0675x - 0.0017$	25–800	0.9988	0.010
Hydroxyacetone	$y = 1.6017x - 0.1099$	75–2400	0.9986	0.066
Acetic acid	$y = 2.3805x + 0.0851$	250–8000	0.9954	0.131
Furfural	$y = 4.3865x + 0.2010$	187.5–6000	0.9973	0.265
Formic acid	$y = 0.3080x + 0.0026$	125–4000	0.9927	0.832
Propionic acid	$y = 2.2079x - 0.0237$	10–320	0.9990	0.176
5-Methyl-2-furfural	$y = 7.6495x + 0.0126$	10–320	0.9966	0.007
Furfuryl alcohol	$y = 3.3525x - 0.0019$	10–320	0.9867	0.024
3-Methyl-1,2-cyclopentanedione	$y = 2.2487x + 0.0064$	1.5–48	0.9922	0.017
Guaiacol	$y = 7.2753x - 0.0005$	1.5–48	0.9921	0.012
Phenol	$y = 5.0193x + 0.0114$	10–320	0.9963	0.030
Pyrrole-2-carboxaldehyde	$y = 6.7498x - 0.0116$	3.125–100	0.9984	0.072
2,6-Dimethoxyphenol	$y = 4.7104x - 0.0173$	1.5–48	0.9994	0.118
Levulinic acid	$y = 1.8504x - 0.1098$	100–3200	0.9919	0.121
3-Hydroxypyridine	$y = 3.0679x - 0.0760$	75–2400	0.9983	0.260
2-Furoic acid	$y = 3.0884x - 0.1056$	20–640	0.9957	0.145
5-Hydroxymethylfurfural	$y = 1.0565x - 0.0095$	187.5–6000	0.9850	0.092
Vanillin	$y = 3.8602x - 0.0342$	12.5–400	0.9947	0.548

Table 6
Precision of peak areas and retention times.

Analyte	Intraday ($n = 6$)		Interday ($n = 6$)	
	Peak area RSD (%)	Retention time RSD (%)	Peak area RSD (%)	Retention time RSD (%)
3-Hydroxy-2-butanone	3.75	0.03	3.81	0.08
Hydroxyacetone	3.89	0.03	4.09	0.07
Acetic acid	4.37	0.03	3.65	0.04
Furfural	1.66	0.02	2.44	0.04
Formic acid	5.13	0.02	2.87	0.04
Propionic acid	4.46	0.02	3.48	0.03
5-Methyl-2-furfural	4.28	0.02	1.10	0.02
Furfuryl alcohol	3.87	0.01	3.32	0.90
3-Methyl-1,2-cyclopentanedione	4.06	0.02	3.80	0.02
Guaiacol	3.79	0.02	3.60	0.02
Phenol	3.92	0.02	3.14	0.02
Pyrrole-2-carboxaldehyde	4.22	0.03	3.66	0.02
2,6-Dimethoxyphenol	3.57	0.01	3.84	0.01
Levulinic acid	5.36	0.01	5.12	0.01
3-Hydroxypyridine	4.78	0.01	5.03	0.03
2-Furoic acid	5.83	0.10	7.17	0.01
5-Hydroxymethylfurfural	6.01	0.01	9.44	0.03
Vanillin	5.49	0.01	2.68	0.01

Table 7
Analytes determined in the corn stover hydrolysate.

Group of compounds	Compounds	Concentration in hydrolysate (g/L)	RSD (%) ($n = 3$)	Recovery[a] (%)
Aliphatic acids	Acetic acid	7.33	5.92	126.1
	Formic acid	4.24	12.10	91.99
	Propionic acid	0.0493	8.16	112.7
	Levulinic acid	2.19	12.21	125.2
Furan derivatives	Furfural	5.73	4.78	67.4
	5-Methyl-2-furfural	0.219	12.73	127.9
	Furfuryl alcohol	0.148	11.92	121.4
	2-Furoic acid	0.454	7.98	92.8
	5-HMF	3.97	9.47	103.3
Phenolic compounds	Guaiacol	0.0103	7.12	114.3
	Phenol	0.0659	11.98	96.7
	2,6-Dimethoxyphenol	0.0224	9.20	121.0
	Vanillin	0.106	5.50	128.0
Others	3-Hydroxy-2-butanone	0.118	4.18	86.6
	Hydroxyacetone	1.18	6.75	124.1
	3-Methyl-1,2-cyclopentanedione	0.0125	3.64	123.5
	Pyrrole-2-carboxaldehyde	0.0254	12.54	92.2
	3-Hydroxypyridine	1.42	10.60	125.5

[a] Recovery calculated by the following equation: $\text{Recovery} = \frac{\text{concentration in spiked sample} - \text{concentration in unspiked sample}}{\text{spiking level}} \times 100\%$.

In the relevant literatures, levulinic acid, acetic acid, 5-hydroxymethyl furfural and furfural have also been reported to be key compounds in dilute acid hydrolysate of lignocellulose [42,43]. In this research, phenolic monomers (phenol, guaiacol, and 2,6-dimethoxy phenol) were taken into account and the total concentration of them was 0.10 g/L. These low molecular phenolic compounds were the most toxic to fermentation despite of their smaller quantities [44]. In addition, 3-hydroxy-2-butanone, hydroxyacetone, and 3-hydroxypyridine were detected with relatively high concentrations (Table 7). However, their potential toxicity to the fermentation of hydrolysate by microorganisms is still not known.

This analysis method is fundamental to overcome the inhibitory problems associated with byproducts in lignocellulosic hydrolysates. On this basis, the formation of byproducts can be minimized through optimization of the pretreatment conditions, and specific detoxification methods can be developed for efficient removal of inhibitory byproducts prior to fermentation.

4. Conclusions

GC–MS equipped with a Deans switch could effectively identify and quantify byproducts in the lignocellulosic hydrolysate of corn stover in direct aqueous injection mode. The GC–MS (SIM) achieved a simultaneous analysis of 18 byproducts, including aliphatic acids, furan derivatives, and phenolic compounds within 45 min. Under optimal chromatographic conditions, the method provided adequate detection limits ranging between 0.007 and 0.832 mg/L and the recovery of the selected byproducts reached 86–128%. Our proposed application of the Deans switch also could extend to the direct qualitative analysis of other trace analytes in different aqueous solution with a MS detector.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (No. 20876078), the Key Program of the National Natural Science Foundation of China (No. 20936002), the United Foundation of NSFC and Guangdong Province (No. U0733001), the National Hi-Tech Research and Development Program of China (No. 2009AA02Z208), the National Key Technology Support Program of China (2011BAD23B03).

References

[1] A. Esteghlalian, A. Hashimoto, J. Fenske, M. Penner, Bioresour. Technol. 59 (1997) 129.

[2] D. Nabarlatz, A. Ebringerová, D. Montané, Carbohydr. Polym. 69 (2007) 20.
[3] A.T.W.M. Hendriks, G. Zeeman, Bioresour. Technol. 100 (2009) 10.
[4] L.T. Fan, Y.H. Lee, M.M. Gharpuray, Adv. Biochem. Eng. Biotechnol. 23 (1982) 157.
[5] L.P. Ramos, Quim. Nova 26 (2003) 863.
[6] E. Palmqvist, B. Hahn-Hägerdal, Bioresour. Technol. 74 (2000) 25.
[7] Z.L. Liu, Appl. Microbiol. Biotechnol. 73 (2006) 27.
[8] L. Olsson, B. Hahn-Hägerdal, Enzyme Microb. Technol. 18 (1996) 312.
[9] E. Palmqvist, B. Hahn-Hägerdal, Bioresour. Technol. 74 (2000) 17.
[10] S.I. Mussatto, I.C. Roberto, Bioresour. Technol. 93 (2004) 1.
[11] S. Larsson, E. Palmqvist, B. Hahn-Hägerdal, C. Tengborg, K. Stenberg, G. Zacchi, N. Nilvebrant, Enzyme Microb. Technol. 24 (1999) 151.
[12] S. Sun, J. Xie, F. Xie, Y. Zong, J. Chromatogr. A 1179 (2008) 89.
[13] F. Bartolozzi, G. Bertazza, D. Bassi, G. Cristoferi, J. Chromatogr. A 758 (1997) 99.
[14] X. Zhu, Q. Su, J. Cai, J. Yang, Anal. Chim. Acta 579 (2006) 88.
[15] F.O. Silva, V. Ferraz, Food Chem. 88 (2004) 609.
[16] P.A.W.V. Hees, J. Dahlén, U.S. Lundström, H. Borén, Talanta 48 (1999) 173.
[17] A.R.B. Quirós, M.A. Lage-Yusty, J. López-Hernández, Talanta 78 (2009) 643.
[18] D. Lefebvre, V. Gabriel, Y. Vayssier, Lebensm. Wiss. u. Technol. 35 (2002) 407.
[19] A. Dupont, C. Egasse, A. Morin, Carbohydr. Polym. 68 (2007) 1.
[20] A.E. Edris, M. Murkovic, B. Siegmund, Food Chem. 104 (2007) 1310.
[21] P. Persson, S. Larsson, L.J. Jönsson, N. Nilvebrant, Biotechnol. Bioeng. 79 (2002) 694.
[22] R. Pecina, P. Burtscher, G. Bonn, O. Bobleter, Fresenius J. Anal. Chem. 325 (1986) 461.
[23] C. Proestos, D. Sereli, M. Komaitis, Food Chem. 95 (2006) 44.
[24] I. Lobo, A.A. Mozeto, Q.B. Cass, Chromatographia 52 (2000) 727.
[25] Y. Zuo, C. Wang, J. Zhan, J. Agric. Food Chem. 50 (2002) 3789.
[26] S. Chen, R.A. Mowery, V.A. Castleberry, G.P.V. Walsum, C.K. Chambliss, J. Chromatogr. A 1104 (2006) 54.
[27] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Tech. Rep. Natl. Renew. Energy Lab. 510 (2008) 42623.
[28] L.N. Sharma, C. Becker, C.K. Chambliss, Biofuels: Methods Protoc. Methods Mol. Biol. 581 (2009) 125.
[29] R.F. Helm, J. Jervis, W.K. Ray, N. Willoughby, B. Irvin, J. Hastie, D.J. Schell, N. Nagle, J. Agric. Food Chem. 58 (2010) 12642.
[30] J.F. Humpula, S.P.S. Chundawat, R. Vismeh, A.D. Jones, V. Balan, B.E. Dale, J. Chromatogr. B 879 (2011) 1018.
[31] J.D. McCurry, Agilent Technologies Publication 5989-1840EN, November 2004.
[32] J.V. Seeley, N.J. Micyus, S.V. Bandurski, S.K. Seeley, J.D. McCurry, Anal. Chem. 79 (2007) 1840.
[33] E.A. Kolbrich, R.H. Lowe, M.A. Huestis, Clin. Chem. 54 (2008) 379.
[34] S.J. Marin, R. Coles, F.M. Urry, G.A. McMillin, J. Chromatogr. B 858 (2007) 59.
[35] T. Gunnar, C. Engblom, K. Ariniemi, J. Chromatogr. A 1166 (2007) 171.
[36] S. Hong, C.M. Duttweiler, A.T. Lemley, J. Chromatogr. A 857 (1999).
[37] Q. Xiang, Y.Y. Lee, R.W. Torget, Appl. Biochem. Biotechnol. 113–116 (2004) 1127.
[38] L. Yan, H. Zhang, J. Chen, Z. Lin, Q. Jin, H. Jia, H. Huang, Bioresour. Technol. 100 (2009) 1803.
[39] Y. Ma, M.D. Hays, J. Chromatogr. A 1200 (2008) 228.
[40] J.J. Fenske, D.A. Griffin, M.M. Penner, J. Ind. Microbiol. Biotechnol. 20 (1998) 364.
[41] M.J. Orts, K.M. Holtman, J.N. Seiber, J. Agric. Food Chem. 56 (2008) 3892.
[42] S.P.S. Chundawat, R. Vismeh, L.N. Sharma, J.F. Humpula, L.C. Sousa, C.K. Chambliss, A.D. Jones, V. Balan, B.E. Dale, Bioresour. Technol. 101 (2010) 8429.
[43] B. Du, L.N. Sharma, C. Becker, S.F. Chen, R.A. Mowery, G.P.V. Walsum, C.K. Chambliss, Biotechnol. Bioeng. 107 (2010) 430.
[44] S. Ando, I. Arai, K. Kiyoto, S. Hanai, J. Ferment. Technol. 64 (1986) 567.