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# High performance thin layer chromatography determination of cellobiosan and levoglucosan in bio-oil obtained by fast pyrolysis of sawdust

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ABSTRACT

In this work, high performance thin layer liquid chromatography (HTPLC) is applied to the determination of sugars in fast pyrolysis liquids (bio-oil) and fractions thereof. The proposed procedure allows the separation of anhydrosugar levoglucosan and cellobiosan, as well as glucose, arabinose, xylose and cellobiose. Pre-treatment and derivatization of samples are not necessary and volatile compounds present in bio-oil do not interfere with sugar analysis. The detrimental effect of the complex bio-oil matrix on columns and detector lifetime is avoided by using disposable HTPLC plates. Prior screening of glucose, present especially in aged and aqueous bio-oil fractions, is required to quantify cellobiosan without interference. Concentrations of levoglucosan and cellobiosan in bio-oil samples obtained from *Pinus radiata* sawdust were ranged between 1.27–2.26% and 0.98–1.96% respectively, while a bio-oil sample obtained from native wood contained a higher levoglucosan concentration.



## 1. Introduction

Bio-oil, the liquid product of fast biomass pyrolysis, is attracting considerable interest as a renewable source of liquid fuels and chemicals. There are several methods for thermal biomass conversion. One of them is fast pyrolysis, which maximizes the yield of this liquid fuel [1]. It is a high-density fuel that can be transported and used by conventional systems like power generation turbines [2]. The biomass is decomposed to generate mostly vapors, aerosols and some charcoal. After cooling and condensation, a dark brown liquid is formed (crude bio-oil), with yields of up to 75 wt% (on a dry-feed basis) [1–5].

Characterization of bio-oil is a challenge and several analytical techniques must be applied to obtain a detailed product distribution which is still incomplete. Only about 40% of bio-oil compounds can be quantified by gas chromatography (GC), especially volatile and thermostable compounds [6,7]. On the other hand, 10–15% polar and nonvolatile compounds have been determined by high performance liquid chromatography (HPLC) [6,7]. Such complexity requires laborious sample pre-treatment, including sequential extractions, and derivatization [7]. However, for the development of bio-oil applications, simple and direct analytical methods for bio-oils and their fractions are preferred.

The 'sugar' fraction of bio oil has particular interest as a fuel and as a source of chemicals. As chemical, levoglucosan (1,5-anhydro-β-D-glucopyranose) and cellobiosan (1,6-anhydro-β-cellobiose) may have pharmaceutical applications, for example, in the synthesis of macrolide antibiotics [8]. The use of anhydrosugars in polymer production, non-ionic surfactants and non-hydrolysable polyglucose has also been described [9,10]. Due to the reactivity and sticking tendency [11], components of the 'sugar fraction' should be separated from the whole bio-oil to improve its fuel properties. Anhydrosugars present in bio-oil can be hydrolysed to cellobiose and glucose [11,12] and be used for ethanol production.

The main compounds in the 'sugar' fraction are levoglucosan (Fig. 1A) and cellobiosan (Fig. 1B) with concentrations between 3–6% and 1–3%, respectively. Low concentrations of glucose, xylose, arabinose and cellobiose have also been reported [6]. The concentrations of levoglucosan and cellobiosan, product of cellulose depolymerization, depend on pyrolysis conditions and the raw material employed, with maximum yields obtained at around 500 °C. Pretreatments of biomass by hydrolysis or demineralization can substantially improve the yields of sugars [13].

For rough determination of 'sugars' in bio-oil, a method based on solvent fractionation of the water soluble fraction and analysis by refractive index has been proposed [11]. Levoglucosan determination has been described using HPLC and especially GC/MS

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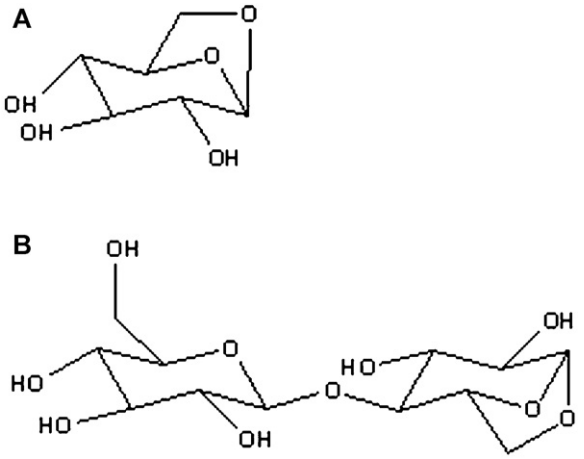


**Fig. 1.** Levoglucosan (A) and cellobiosan (B) chemical structures.

methods [14,15]. Identification of cellobiosan has been described using GC/MS and HPLC in products of pyrolysate cellulose matrix, using spectra libraries [16,17]. Silylation using TMS of bio-oil samples has also been tested for cellobiosan detection by GC/MS [18]. However, no quantification of sugars in bio-oil has been previously described.

Other sugars, like monosaccharides, disaccharides and polysaccharides have of course been determined in different matrices (e.g., food, drugs and human fluids) using high performance thin layer chromatography (HPTLC) [19–23]. However, this technique has not been used extensively for bio-oil analysis, or for anhydrosugar separation. The content of arabinose, galactose, glucose, mannose and xylose has been determined and compared with acid hydrolysis products of woods by three different chromatographic methods [24]. These are borate complex anion-exchange chromatography, anion-exchange chromatography in NaOH medium and HPTLC. Determination of cellobiosan and levoglucosan by the latter has not been described before.

The main aim of this work is to determine sugars, especially anhydrosugars in bio-oil and fractions thereof, by using a HPTLC technique. This offers great advantages in biomass pyrolysis research, especially considering that bio-oils are complex matrices with different kinds of compounds, some of which can be retained in the HPLC column and damage it. For GC analysis, sugars need complex derivatization procedures. On the other hand, bio-oil has many volatile compounds that will not remain on the plate after sample application and will not interfere with sugar separation. HPTLC has the additional advantage over HPLC that the separation layer (the plate) is used just once, so compounds that are irreversibly bonded to the plate are not important. Because the separated analyte remains on the plate after chromatography, multiple development procedures that definitely improve separation can be used, giving reproducible and confident quantitative results, especially if, as in this work, the Automatic Development Chamber (ADC2) is used.

## 2. Materials and methods

### 2.1. Reagents and standard

Levoglucosan (1,6-anhydro-β-D-glucopyranose) was obtained from Merck (Hohenbrunn, Germany). Cellobiosan (1,6-anhydro-β-cellobiose) from Sussex Research Laboratories Inc. (Ottawa, Canada). D(+)-Cellobiose was obtained from Sigma–Aldrich. L(+)-Arabinose, D(+)-xylose, D(+)-glucose, methanol (liquid chromatography grade), acetonitrile (liquid chromatography grade), 1-butanol (Lichrosolv), aniline p.a, diphenylamine p.a, di-potassium phosphate anhydro p.a, formic acid (98–100%) and orthophosphoric acid (85%) p.a, were all obtained from Merck (Darmstadt, Germany). Deionized water (18 mΩ) was produced with a water purification system Millipore Milli-Q (Bedford, MA, USA).

### 2.2. Instrumentation

The HPTLC system was constituted by a Scanner 3 spectrodensitometer, running winCATS 1.4.3 software, an automatic application band device ATS 4 and an automatic developing chamber ADC 2, all from CAMAG (Muttenz, Switzerland). Chromatographic plates were 20 cm × 10 cm silica gel 60 F254 HPTLC plates (extra thin) from Merck (Darmstadt, Germany), impregnated with phosphate. Glucose screening method used the same HPTLC not impregnated plates. CAMAG TLC Immersion Device III and a CAMAG TLC Plate Heather III were used for plate treatment and visualization. Evaluation of different mobile phases used in separation of cellobiosan from glucose was carried out using a HPTLC Vario Chamber.

### 2.3. Standard solutions and bio-oil samples

Stock solutions of sugars were prepared in methanol/water 70/30 (v/v) at 1.0 mg mL$^{-1}$. Bio-oil samples and extracts were diluted 1:25 (v/v) in methanol. No other sample treatment was required before HPTLC analysis.

The samples were weighted on analytical balance and dissolved in methanol. This last step was carried out at the moment of analysis, because methanol can react over time with compounds present in bio-oil. Generally, sugars can react with acids, esters or alcohols by acid catalysis [25] and with bio-oil at pH around 2–3, can react during storage of diluted samples.

Bio-oil samples (1, 2, 3 and 4) were produced in a bench-scale pyrolysis plant at the Metallurgical Engineering Department of the University of Concepción. In each run, oven-dry sawdust was fed into a fluidized bed reactor using nitrogen and pyrolyzed in contact with hot sand. After removing char, bio-oil was condensed and collected in two catch pots. The first one collected was the main liquid product after the condenser and cyclone; the second one collected was bio-oil drops trapped in a demister. Bio-oil samples 1 and 4 were obtained by mixing the two liquid products, while bio-oils 2 and 3 were samples of the two liquids. Table 1 shows a summary of some of the physico-chemical characteristics of the obtained bio-oils.

In addition, three bio-oil fractions were prepared from bio-oil 4 for 'sugar' analysis in different matrices: a water insoluble

**Table 1**
Physico-chemical properties of analyzed bio-oil samples obtained by pyrolysis at 500 °C.

| Bio-oil samples[a] | Sawdust source | Viscosity (at 40 °C) (cSt) | Density (at 20 °C) (g/cm$^3$) | Total acids (mg KOH/g) |
|---|---|---|---|---|
| Bio-oil 1 | *Pinus radiata* | 13.6 | 1.18 | 54 |
| Bio-oil 2 | *Pinus radiata* | 11.4 | 1.17 | 48 |
| Bio-oil 3 | *Pinus radiata* | 2.4 | 1.11 | 45 |
| Bio-oil 4 | Native wood | 7.7 | 1.18 | 66 |

[a] Bio-oil 2 corresponds to the main liquid product collected in catch pot A (after condenser and cyclones); bio-oil 3 is the liquid product received in catch pot B (demister). Bio-oils 1 and 4 are the mixtures of products collected in catch pots A and B.

lignin derived precipitate, an aqueous phase and an organic extract obtained as described below.

*2.3.1. Bio-oil extraction with n-butanol*

100 mL of bio-oil 4 was extracted with 50 mL of *n*-butanol and 50 mL of water. The heavier, water-rich fraction was extracted again with the same mixture, and another time with 50 mL of *n*-butanol. The water-rich fraction (water phase) and the mixture of 3 *n*-butanol extracts (butanol-phase) were analyzed.

*2.3.2. Pyrolytic lignin*

20 mL of bio-oil 4 was very slowly dispersed in 200 mL cold water (5 °C) with the help of an IKA T-25 Ultra-Turrax at 6000 rpm. The precipitate or "pyrolytic lignin", was filtered off and the filtrate was analyzed.

*2.4. HPTLC quantification of main sugars*

HPTLC silica–gel plates were impregnated with anhydrous dipotassium phosphate (0.2 M)/methanol 1:1 (v/v), using the TLC Immersion Device with a dipping speed of 2 cm $s^{-1}$ and dipping time of 8 s. The drying was carried out at 120 °C for 20 min. Sample application was done as 8 mm bands, using 0.1–1 μL of the standard solution (100–800 ng per band) in the calibration. The chromatographic procedure was a multiple development (three times) with water/acetonitrile 20/80 (v/v) as mobile phase [26]. The development distance was 70 mm from the lower edge of the plate. A solution containing 1.2 g of aniline, 1.2 g of diphenylamine, 10 mL of phosphoric acid and 100 mL of methanol was used as chromogenic reagent [27]. Post-chromatographic derivatization on silica–gel was performed with the TLC Immersion Device using the same condition as described before. Sugars were visible after 15 min in the plate heater at 120 °C. Detection was performed by scanning the plate at 520 nm in the reflectance/absorbance mode.

Different mobile phases were evaluated in order to detect glucose, especially in aged bio-oil samples. Finally the separation was carried out on plates without impregnation and using butanol/formic acid 45:5 (v/v) [28] as the mobile phase. Development distance, chromogenic reagent and detection wavelength were as described in the preceding paragraph for the other sugars.

## 3. Results and discussion

*3.1. Separation of main sugars and calibration curves*

Fig. 2 shows the HPTLC plate of bio-oil 1 and sugar standards using the methodology described in Section 2.4. The calibration curves for levoglucosan and cellobiosan were prepared between 100 and 800 ng by band, 50–400 ng by band for xylose and cellobiose, and 50–300 ng by band for arabinose. The chromatographic development was carried out using the procedure described in Section 2.4. As can be seen, the five analytes are adequately separated with $R_f$ 0.29, 0.35, 0.42, 0.49 and 0.69 for cellobiose, cellobiosan, arabinose, xylose and levoglucosan, respectively. No other interfering compounds from the bio-oil matrix are observed, allowing the quantification of these sugars without major sample pretreatment steps, as required in GC or HPLC.

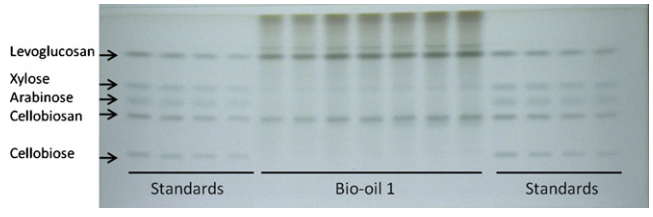


**Fig. 2.** HPTLC plate of bio-oil 1 and sugar standards using acetonitrile/water 80/20 (v/v) as mobile phase.

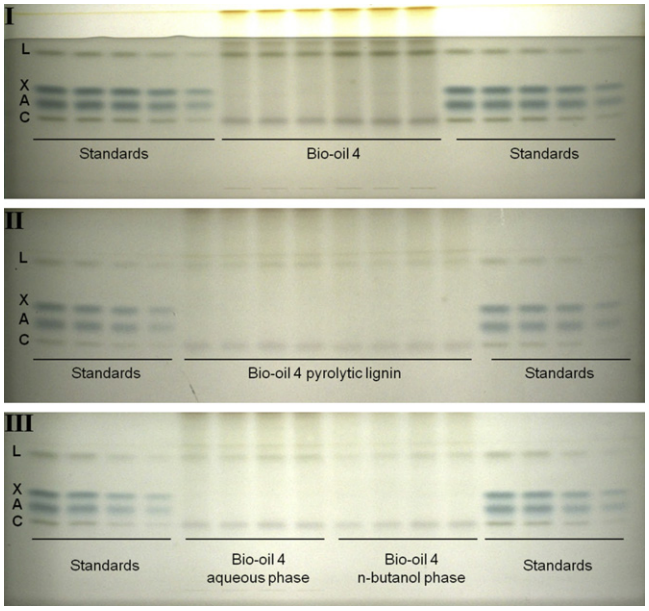


**Fig. 3.** HPTLC plates of bio-oil 4 and their fractions using acetonitrile/water as mobile phase. (I) bio-oil 4; (II) pyrolytic lignin; (III) *n*-butanol and aqueous phase. Standards: cellobiosan (C), arabinose (A), levoglucosan (L) and xylose (X).

In Fig. 3 are included the HPTLC plates of the fraction obtained from bio-oil 4. It is possible to detect the studied sugars in these fractions with the optimized method using the procedure described in Section 2.4. Therefore, the developed method can also be used for different fractions of bio-oil.

Linearity, detection and quantification limits and intermediate precision (the latter measured with a bio-oil sample) are summarized in Table 2. The detection limits obtained for levoglucosan and cellobiosan were acceptable, considering that the reported concentrations of these sugars in bio-oils from sawdust are around 1–6 wt% [2].

The determination of anhydrosugars in crude bio-oil and fractions can be affected by the presence of glucose, produced by first-order hydrolysis reactions of levoglucosan and cellobiosan. The decay in anhydrosugar concentrations was notorious in aqueous fractions of bio-oil and aged bio-oil samples. Hence, for quantification of the main sugars in bio-oil and aqueous fractions, the presence of glucose must be established. This separation has not been studied before using any chromatographic method. The first results of HPTLC analysis showed good separation between levoglucosan and cellobiosan, but not between cellobiosan and glucose. Different mobile phases were studied for HPTLC separation of cellobiosan from glucose (butanol/boric acid (100 mg/20 mL water/iso-propanol 30:50:10 (v/v) [25]; ethyl acetate/hexane 1:9 (v/v) [29]; benzene/acetone 10:1 (v/v) [30]; chloroform/methanol/water/acetic acid 30:12:4:5 (v/v) [31] and butanol/formic acid 45:5 (v/v) [28]). Finally, separation of both analytes was achieved by using a mobile phase of butanol/formic acid 45:5 (v/v). In Fig. 4 the separation of cellobiosan and glucose in a sample of crude bio-oil 2 using these conditions is shown. The screening of glucose in bio-oil 4 and different fractions is shown in Fig. 5. In Fig. 6 the glucose screening is shown in the presence of all sugar standards. Under these conditions, xylose and levoglucosan are not separated.

Considering that this procedure is effective only to separate glucose from cellobiosan, it was used just for screening purposes,

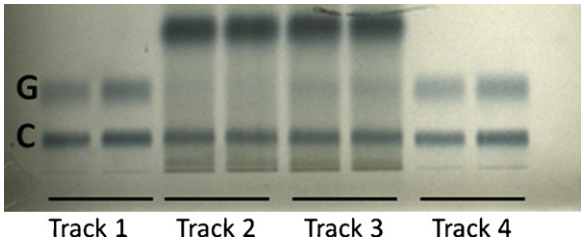


**Fig. 4.** HPTLC plate of bio-oil samples, cellobiosan and glucose standards using butanol/formic acid as mobile phase. Cellobiosan (C) and glucose (G). Tracks 1 and 4 cellobiosan and glucose standards. Track 2 bio-oil 2 without glucose. Track 3 bio-oil 2 with glucose added.

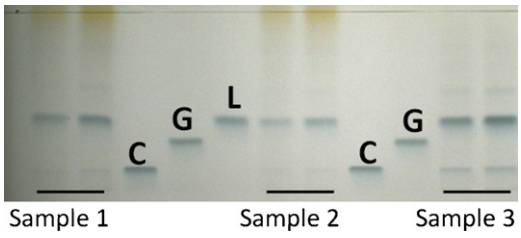


**Fig. 5.** Screening of different bio-oil samples for glucose detection. Cellobiosan (C), glucose (G) and levoglucosan (L). Samples 1, 2 and 3 correspond to bio-oil 4, pyrolytic lignin and bio-oil aqueous phase respectively.

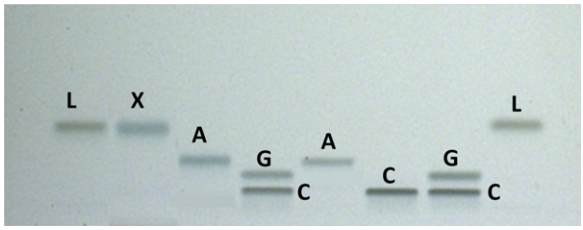


**Fig. 6.** HPTLC plate of sugars standards using butanol/formic acid as mobile phase. Cellobiosan (C), glucose (G), arabinose (A), levoglucosan (L) and xylose (X).

avoiding the overestimation of cellobiosan due to the presence of glucose. Based on this fact, Fig. 7 shows the scheme adopted for the determination of principal sugars in bio-oil samples.

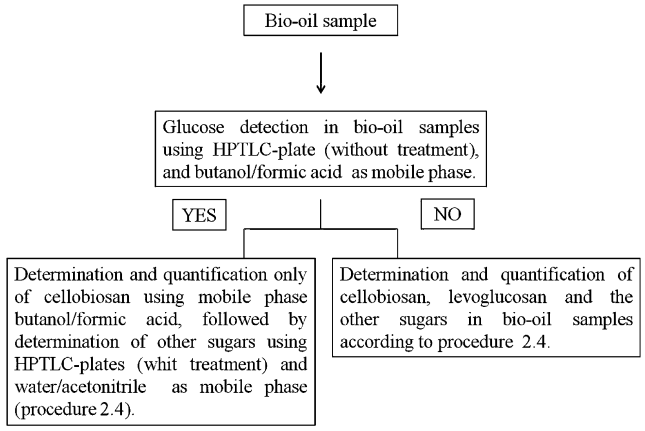


**Fig. 7.** Proposed scheme for the determination of principal sugars in bio-oil samples.

### 3.2. Quantification of sugars in bio-oil samples and fractions

Table 3 shows the results obtained by the proposed HPTLC method for seven samples of fresh bio-oil and bio-oil fractions. Different samples were selected for analysis, including bio-oils produced from soft- and hardwood sawdust, bio-oils collected at different points in the pyrolysis condensation train, and samples from exploratory bio-oil fractionations.

Analysis revealed no presence of glucose, cellobiose, or arabinose in the freshly produced bio-oils. Analytical signals for xylose were observed, but their values were below the detection limit of the methodology (see Fig. 2). However, in aged bio-oil samples, detectable amounts of monosaccharides were found (results not shown).

The raw material for the production of bio-oils 1–3 was sawdust of *Pinus radiata*, and for bio-oil 4 a mixture of native woods was used (see Table 1). The concentration of levoglucosan was higher for bio-oil 4, which is in accordance with those levels reported by Ingram et al., that shows higher concentrations of this sugar in hardwood (oak wood) than in softwood [32].

The bio-oils 2 and 3 were collected at different steps of the pyrolysis condensation train: bio oil 2 at the exit of the cyclone after the condenser, and bio oil 3 at the bottom of the demister. The presence of anhydrosugars in bio-oil 3 shows that high-molecular-weight compounds are not condensed and are not trapped effectively in the condenser and these are carried over to the demister. Pyrolysis

**Table 2**
Analytical parameters for HPTLC sugar determination in bio-oil.

| Sugars | Linear range (ng) | Detection limit (ng) | Quantification limit (ng) | Intermediate precision[a] (RSD ($n$ = 3)) |
|---|---|---|---|---|
| Levoglucosan | 100–800 | 60 | 180 | 11% |
| Cellobiosan | 100–700 | 80 | 240 | 20% |
| Xylose | 50–400 | 16 | 48 | – |
| Arabinose | 50–300 | 14 | 42 | – |
| Cellobiose | 50–300 | 18 | 59 | – |

[a] The intermediate precision was determined using three different bio-oil samples in triplicate, at different days and with different HPTLC plates.

**Table 3**
Sugar concentrations in fresh bio-oil samples and extracts.

| Samples | Glucose | Levoglucosan (wt%) | Cellobiosan (wt%) | Xylose (wt%) | Arabinose (wt%) |
|---|---|---|---|---|---|
| Bio-oil 1 | ND[a] | 1.27 | 1.46 | ND | ND |
| Bio-oil 2 | ND | 1.90 | 1.99 | ND | ND |
| Bio-oil 3 | ND | 1.68 | 0.98 | ND | ND |
| Bio-oil 4 | ND | 2.26 | 1.40 | ND | ND |
| Bio-oil 4 aqueous phase | ND | 1.81 | 0.93 | ND | ND |
| Bio-oil 4 *n*-butanol/phase | ND | 0.78 | 0.82 | ND | ND |
| Bio-oil 4 pyrolytic lignin | ND | 0.75 | 0.88 | ND | ND |

[a] ND, not detected; wt%, weight/weight percent.

products tend to form aerosols difficult to trap successfully. In this case, no final electrostatic precipitator has been used.

Common bio-oil fractionation schemes start by removing high-molecular-weight compounds derived from lignin by the addition of water and precipitation. A heavy, viscous organic fraction settles down and sticks to the walls of the recipient. However, if an excess of cold water and a homogenizer are used, a fine precipitate can be collected. In Table 3, the fine precipitate (bio-oil 4 pyrolytic lignin) displays a similar concentration of anhydrosugars, compared to the source bio-oil. As this fraction accounts 19–23 wt% of the whole bio-oil, a similar percentage of anhydrosugars remains trapped in the pyrolytic lignin. Re-suspension in water and repeated filtration and washing may be necessary to improve the separation of anhydrosugars from pyrolytic lignin.

An alternative bio-oil fractionation scheme involves an organic polar solvent that dissolves the hydrophobic lignin compounds, giving a lighter organic phase and a heavier water fraction. In the case of bio-oil extracted with *n*-butanol/water, the yield of pyrolytic lignin in the butanol phase, determined by the same method of dispersion and precipitation in an excess of cold water (1:10 v), is almost the same as the yield from bio-oil. Therefore, the high-molecular-weight lignin-derived compounds end up effectively in the butanol phase, as intended; but anhydrosugars as can be seen from Table 3, are also significantly extracted into the butanol phase.

Our results show that a high percentage of sugars still remain in the pyrolytic lignin fraction, obtained either by dispersion using water or organic polar solvent, producing both treatments similar sugar levels. Another dispersion step, using water, followed by filtration and washing could allow the separation between anhydrosugar and pyrolytic lignin.

## 4. Conclusions

The HPTLC technique displays a major advantage in the analysis of bio-oil samples than other expensive or laborious procedures using HPLC or GC: no sample pre-treatment and disposable HPTLC plates providing a new separation layer for each sample. The proposed HPTLC method shows adequate analytical parameters for the quantification of main sugars in bio-oil samples and their fractions. In addition, the same methodology can be used to quantify the main sugars in different bio-oil extracts (butanolic, pyrolytic lignin and aqueous extract) with adequate intermediate precision. In all studied bio-oils, cellobiosan and levoglucosan were detected but not xylose, arabinose and cellobiose. On the other hand, glucose screening of bio-oil samples is needed, because of its interference with cellobiosan, especially for aged bio-oil samples. A separation using butanol/formic acid as mobile phase allows for this purpose. Considering this fact, a scheme for the determination of principal sugars in bio-oil samples is proposed. Separation of anhydrosugars from pyrolytic lignin is complex. To achieve their separation, an additional resuspension in water may be necessary.

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## References

[1] T. Bridgwater, J. Sci. Food Agric. 86 (2006) 1755.
[2] New Hampshire Bio-oil Opportunity Analysis, New Hampshire Office of Energy & Planning, Innovative Natural Resource Solutions LLC, 2004, www.nh.gov/oep.
[3] Z. Qi, C. Jie, W. Tiejun, X. Ying, Energy Convers. Manage. 48 (2007) 87.
[4] J.P. Diebold, National Renewable Energy Laboratory, NREL/SR-570-27613, 2000.
[5] D. Mohan, C. Pittman, P. Steele, Energy Fuels 20 (2006) 848.
[6] A. Oasmaa, D. Meier, J. Anal. Appl. Pyrol. 73 (2005) 323.
[7] R. Bayerbach, J. Anal. Appl. Pyrol. 85 (2009) 98.
[8] S. Helle, N. Bennett, K. Lau, J. Matsui, S. Duff, Carbohydr. Res. 342 (2007) 2365.
[9] A. Oasmaa, E. Kuoppala, Energy Fuels 22 (2008) 4245.
[10] A.V. Bridgwater, Fast Pyrolysis of Biomass: A Handbook, vol. 2, Antony Rowe Ltd., Chippenham, UK, 2002 (Chapter 10).
[11] N. Kochetkov, N. Yashunsky, A. Svirido, M. Ermolenko, Carbohydr. Res. 200 (1990) 209.
[12] E. Olson, B. Freel. US-Patent N° 2007/0125369 A1 (2007).
[13] D. Radlein, J. Piskorz, D.S. Scott, J. Anal. Appl. Pyrol. 19 (1991) 41.
[14] D. Simpson, R. Dills, B. Katz, D. Kalman, J. Air Waste Manag. Assoc. 54 (2004) 689.
[15] R. Dhammapala, C. Claiborn, J. Jimenez, J. Corkill, B. Gullett, C. Simpson, M. Paulsen, Atmos. Environ. 41 (2007) 2660.
[16] J. Lomax, J.M. Commandeur, P. Arisz, J. Boon, J. Anal. Appl. Pyrol. 19 (1991) 65.
[17] D. Radlein, A. Grinshpun, J. Piskorz, D. Scott, J. Anal. Appl. Pyrol. 12 (1987) 39.
[18] O. Boutin, PyNews Pyrol. Network 5 (1998) 14.
[19] G. Morlock, M. Vega, J. Planar Chromatogr. 20 (2007) 411.
[20] A. Zuñiga, P. Inostroza, M. Vega, L. Bravo, L. Corcuera, Antarct. Sci. 15 (2003) 483.
[21] L.W. Doner, Chromatographia 53 (2001) 579.
[22] T. Ranganathan, P. Kulkarni, Food Chem. 77 (2002) 263.
[23] Q. Wei Liang, K. Zeeshan, J. Food Compos. Anal. 21 (2008) 78.
[24] A. Uremovic, T. Dokk, J. Schuseil, B. Saake, A. Borchmann, A. Herrmann, J. Puls, Holz als Roh-und Werkstoff 52 (1994) 347.
[25] R. Klaus, J. Ripphahn, J. Chromatogr. 244 (1982) 99.
[26] C. Conaway, B. Fried, J. Sherma, J. Planar Chromatogr. 8 (1995) 184.
[27] H. Jork, W. Funk, W. Fischer, H. Wimmer, Thin-layer Chromatography: Reagents and Detection Methods, vol. 1a, VCH, Verlagsgesellschaft, Germany, 1999, p. 179.
[28] N. Perisic-Janjic, M. Popovic, S. Radosavljevic, Symposium on TLC with Special Emphasis on OPLC, Szeged, vol. 68, 1984.
[29] D. Alperin, H. Carminatti, R. Couso, J. Chromatogr. 265 (1983) 193.
[30] I. Cerny, T. Trnka, M. Cerny, Chem. Commun. 48 (1983) 2386.
[31] Z. Ping-Ping, L. Bao-Ming, H. Li-Yi, Acta Pharmacol. Sin. 22 (1987) 70.
[32] L. Ingram, D. Mohan, M. Bricka, Energy Fuels 22 (2008) 614.