Analytical Methods

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2015, **7**, 7866

Simultaneous determination of acid-soluble biomass-derived compounds using high performance anion exchange chromatography coupled with pulsed amperometric detection†

N. Anders,*[a] H. Humann,[a] B. Langhans[a] and A. C. Spieß[ab]

A high performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) method using a CarboPac™ PA100 column for the simultaneous determination of 25 soluble compounds originating from all polymer classes (cellulose, hemicellulose, lignin and pectin) in biomass hydrolyzates was developed and validated. The method was optimized for resolution varying the column oven temperature (in the range of 30–50 °C) and the eluents (100 mM NaOH, 200 mM NaOH, 100 mM NaOH/500 mM NaOAc and water). The optimized method used gradient elution at 40 °C and had 70 min duration for one run. The detection limits ranged between 0.14 mg L^{-1} for both 2,6-dimethoxyphenol and 3,5-dimethoxy-4-hydroxybenzaldehyde and 21.9 mg L^{-1} for 4-methoxybenzyl alcohol, linearity was always >0.99, and sensitivity ranged between 0.0023 (nC min)/(mg L^{-1}) for glucuronic acid and 8.4 (nC min)/(mg L^{-1}) for 3,5-dimethoxy-4-hydroxybenzaldehyde. In contrast to the established photometric lignin measurement the HPAEC-PAD method allows for a distinction between several acid-soluble lignin compounds. The method was applied to 17 different biomass hydrolyzates and displayed the capability to quantify a wide range of soluble products covering very different hydrolyzate compositions.

Received 27th May 2015
Accepted 1st August 2015

DOI: 10.1039/c5ay01371b

www.rsc.org/methods

Introduction

The depletion of fossil raw materials motivates mankind to think about alternatives, in particular biomass as a renewable and CO_2-neutral carbon resource. The perfect feedstock for bulk chemicals or fuels should have a high content of saccharifiable and easily degradable carbohydrates, namely cellulose and hemicellulose. Beyond these carbohydrate polymers, natural feedstocks contain lignin, pectin, other organic compounds and salts.[1–6] It is possible to isolate cellulose, hemicellulose and lignin from wood or grain.[2,3,5,6] Challenges for the isolation arise from the composition, as for example orange peels consist of but are not limited to 10 to 37% cellulose, 10 to 15% hemicellulose, *ca.* 6% lignin and 28 to 40% pectin.[1,4] In order to decide whether the feedstock is suitable for biomass hydrolysis, a complete characterization of the feedstock is essential.

The current standard for feedstock analysis is a two-step acid hydrolysis procedure originally proposed by Saeman or the optimized procedure published as NREL/TP-510-42618.[7,8] This procedure enables the determination of cellulose and hemicellulose based on their monomeric compounds. Lignin can be calculated as acid-soluble lignin (ASL) and as acid-insoluble lignin (AIL). Due to the different chemical structures and polarities, the biomass-derived compounds require various analytical devices such as a photometer and a high-performance liquid chromatography coupled with refractometric index detection (HPLC-RI). HPLC-RI analysis allows for a complete analysis of polar and unpolar compounds using various chromatographic columns. The main disadvantages of the established procedures are the low information content of a photometric measurement of a compound mixture, the time-consuming procedure which requires complex equipment, the need for calibrating several methods and for a versatile know-how. Those have to be overcome in order to gain more information in an adequate time.

In order to minimize the analytical effort for screening different feedstocks, one analytical device which allows for the estimation of as many as possible of the soluble cellulose, hemicellulose, lignin and pectin-derived components is desirable. As a separation of the monosaccharides derived from cellulose and hemicellulose is required, several chromatographic methods come into question. Liquid chromatography (LC) would be favored over gas chromatography (GC) since no additional derivatization step is required to bring the

[a]*AVT-Enzyme Process Technology, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany. E-mail: nico.anders@avt.rwth-aachen.de*

[b]*DWI – Leibniz Institute for Interactive Materials, Forckenbeckstraße 50, 52074 Aachen, Germany*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ay01371b

monosaccharides into the mobile phase.[9,10] Within the LC methods, the high performance anion exchange chromatography (HPAEC) has been validated for the simultaneous determination of selected mono- and oligosaccharides and uronic acids and is frequently applied for these groups of compounds.[2,10–16] Additionally, the electrochemical pulsed amperometric detection (PAD) principle allows for a selective and sensitive quantification of diverse oxidizable or reducible compounds.[17] As a result, compounds eluting at similar retention times will not interfere with the quantification of mono- and oligosaccharides if these compounds are not electroactive. Therefore, HPAEC-PAD methods are often used to quantify heterogeneous biomass hydrolyzates for sugars.[2,18,19]

The deprotonation of hydroxyl groups in strong alkaline media leads to the assumption that not only mono- and oligosaccharides and uronic acids, but also ASL compounds containing a hydroxyl group should be determinable using the HPAEC-PAD system. This would allow for the separation and quantification of individual ASL compounds and, therefore, a complete characterization of the soluble depolymerization compounds from biomass hydrolyzates using only one device. Accordingly, this study proposes for the first time the development of an analytical method based on HPAEC-PAD equipped with a CarboPac™ 100 PA column which can be used for the separation, identification and quantification of acid-soluble cellulose-, hemicellulose-, lignin- and pectin-derived compounds in biomass hydrolyzates. In order to attain this objective the column temperature (30–50 °C) and the eluents (sodium acetate, sodium hydroxide and water) were varied. The analytical method described here has been validated and then applied to characterize 17 various biomasses.

Results and discussion

Optimization of separation conditions for soluble biomass derived compounds

The HPAEC-PAD allows for an adaptation of the sodium hydroxide concentration. The sodium hydroxide concentration will influence the retention times of different substance classes depending on their acid strength. Thus, an investigation of the eluent composition becomes necessary in order to separate the compounds. Furthermore, the temperature was investigated, since it influences the separation and the acid base reaction.

As the eluent flow correlates strongly to the overall method time only an investigation of higher flows is acceptable in order to avoid longer retention times. High eluent flows are challenging to handle as modern devices are programmed to strictly reduce the flow after exceeding a specific pressure value. Thus, we decided to use a fixed flow rate avoiding long retention times and the risk of the abort of the automated sequence.

As acid hydrolysis leads to a range of compounds, mono- and oligosaccharides, as well as uronic acids, and the sugar degradation product 5-HMF were investigated. Beyond that, the quantification of the following ASL-derived compounds using HPAEC-PAD is attempted for the first time: 4-allyl-2-methoxyphenol, 2,6-dimethoxyphenol, 3,5-dimethoxy-4-hydroxybenzaldehyde and 2-methoxyphenol which are considered as relevant lignin-derived compounds.[20–22]

For the separation behavior of the acid-soluble compounds the column oven temperature (30–50 °C) and the eluent composition (water, 100 mM NaOH, 200 mM NaOH, and 100 mM NaOH/500 mM NaOAc) were varied in order to yield a good resolution of the typical hydrolyzate compound groups and the new lignin standards, indicated by their retention time (Fig. 1).

Due to the fact that oligosaccharides have longer retention times compared to monosaccharides, compounds with a lower number of hydroxyl groups seem to have shorter retention times. On the basis of this assumption the ion strength of the eluent was increased in order to clearly separate the ASL compounds, 5-HMF, monosaccharides, oligosaccharides and uronic acids. Fig. 1 shows that ASL compounds elute first. Five ASL compounds (2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylalcohol, 3,4-dimethoxybenzaldehyde, 4-methoxybenzylalcohol and 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (lignin model compound, LMC)) form a first compound group together with 5-HMF (Fig. 1A and B). Surprisingly, the mobile phase composition in the investigated range of 50–90% (v/v) water with 50–10% (v/v) 100 mM NaOH has only a minor influence on the retention time of both the sugar degradation product 5-HMF and the acid-soluble compounds from lignin (see Fig. 1A). The low retention time of the first compound group (Fig. 1A) can be explained on the one hand by the low pK_a values of the alcohols and, on the other hand, by the structural differences in the position of the hydroxyl group (structures see in ESI Tables S1 and 2†) which both lead to a low interaction of the substances with the column material.

Due to the lower pK_a values, the monosaccharides have a slightly higher, but partially overlapping, retention time in comparison to the first group of fast-eluting ASL compounds with 5-HMF (Fig. 1A–D). As monosaccharides are weak acids they are deprotonated easily by basic eluents. Caused by the low differences in the acid strength, the separation of these monosaccharides can be improved by using a low concentration of sodium hydroxide in the eluent. Notably, the separation of galactose and glucose can be obtained at an eluent composition of 85% water and 15% sodium hydroxide (Fig. 1C).

Due to the higher interaction of the oligosaccharides and the uronic acids with the column material the gradient was changed from this water and sodium hydroxide mixture to a sodium hydroxide and sodium acetate mixture. Fig. 1E shows a decrease of the retention time with increasing sodium acetate composition. The best separation of oligosaccharides was achieved using between 15 and 25% of the 500 mM sodium acetate eluent (Fig. 1E). A similar behavior could be observed for the uronic acids. Here, the retention time decreases with increasing sodium acetate concentration, too. However, the separation of galacturonic acid and glucuronic acid increases with increasing sodium acetate content (Fig. 1G). Therefore, the concentration of the eluent 500 mM sodium acetate is increased to 45%.

Contrary to expectations some ASL compounds have retention times higher than 25 minutes (Fig. 1I and J). Thus, more than just acid strength and the number of hydroxyl groups seem to be the reason for the late elution. Further investigations of

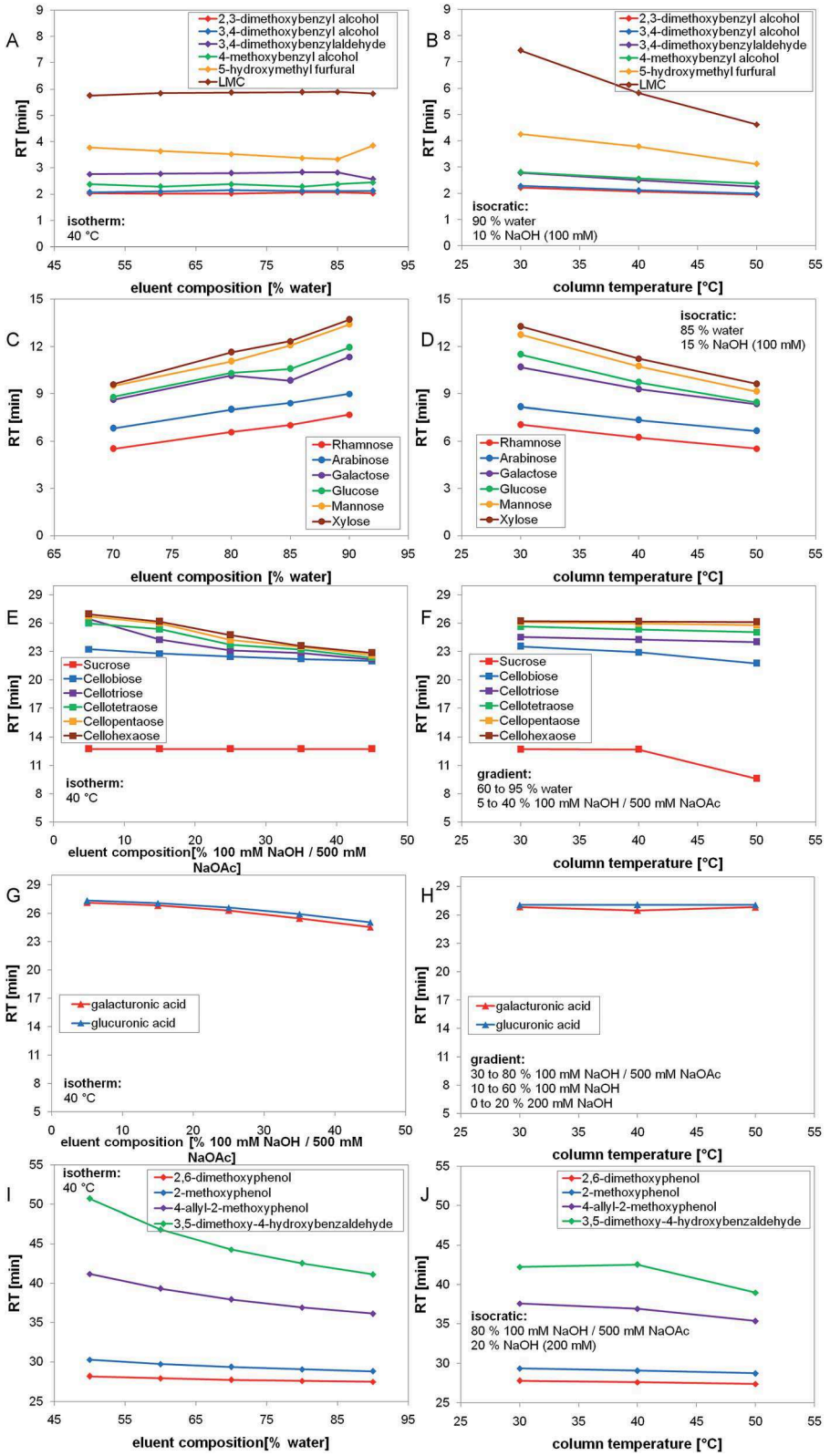

Fig. 1 Effect of eluent composition (A, C, E, G, I) and column temperature (B, D, F, H, J) on the retention time of acid-soluble biomass compound groups. The influence of the retention time for acid-soluble lignin compounds (A, B, I and J), monosaccharides (C and D), soluble oligosaccharides (E and F) and uronic acids (G and H) was investigated for both column temperature (in the range of 30–50 °C) and the eluent composition (water, sodium hydroxide and sodium acetate).

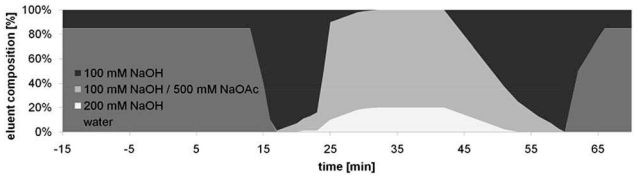

Fig. 2 HPAEC-PAD gradient. Gradient of four eluents (100 mM NaOH, 100 mM NaOH and 500 mM NaOAc, 200 mM NaOH and water) for the simultaneous determination of mono- and oligosaccharides, 5-HMF, uronic acids and acid-soluble lignin compounds.

the behavior of this last compound class containing 2,6-dimethoxyphenol, 2-methoxyphenol, 3,5-dimethoxy-4-hydroxybenzaldehyde and 4-allyl-2-methoxyphenol in the eluent need to be performed in order to investigate this effect.

In order to elute also undetectable organic acids, both the sodium acetate composition and the sodium hydroxide composition are further increased and, thus, the chromatographic run is extended.

The optimization of the temperature in the range of 30–50 °C is shown in Fig. 1B–J. The retention time and the separation between the fast-eluting ASL compounds and 5-HMF decrease with increasing column temperature. The same can be observed for the monosaccharides (see Fig. 1B and D). However, the retention time and the separation of oligosaccharides slightly decrease with increasing column temperature (Fig. 1F). Furthermore, the uronic acids show the best separation at a column temperature of 40 °C (Fig. 1H). Since at no condition an optimal separation of all substance classes could be accomplished, the column temperature was fixed to 40 °C as a reasonable compromise. The final gradient is shown in Fig. 2.

By varying mainly the eluent composition it could be shown that not only mono- and oligosaccharides, their degradation products and uronic acids, but also the important ASL compounds can be retained and separated by the column and measured by the detection system. In principle, all compounds which can be deprotonated under highly alkaline conditions are retained by the column. Subsequently, the deprotonated compounds are oxidized in the detector and, thus, a measurable signal is formed. The fact that the ASL compounds are detectable suggests that the mechanism also applies to these compounds.

The newly developed method leads to a clear separation of biomass derived soluble compounds, namely commercially available ASL compounds, sugar degradation products, mono- and oligosaccharides and uronic acids (ESI Fig. S1†).

Method validation

The HPAEC method for the quantification of biomass hydrolyzate compounds was validated based on the following quantitative criteria: linearity, sensitivity, detection limit and standard deviation.

The linearity of the HPAEC-PAD method was tested in a range between 5 mg L^{-1} and up to 200 mg L^{-1} depending on the solubility of the compound, resulting in an R^2 value > 0.99 for all compounds (Table 1). Thus, a proportional dependence of the signal from the substance concentration in the investigated range could be proven. Furthermore, the standard deviation of the procedure as well as the limit of detection (LOD) were calculated.[23] For further reference to other methods for the determination of the limit of detection, the noise of the HPAEC-PAD method was determined to 0.0091 nC. These values allow for an interpretation and comparison of different methods. Table 1 shows that the sensitivity varies in the range of 0.0023 (nC min)/(mg L^{-1}) for glucuronic acid and 8.4 (nC min)/(mg L^{-1}) for 3,5-dimethoxy-4-hydroxybenzaldehyde, the standard deviation of the procedure in a range of 0.05 mg L^{-1} for 3,5-dimethoxy-4-hydroxybenzaldehyde and 7.28 mg L^{-1} for 4-methoxybenzyl alcohol and the LOD in a range of 0.14 mg L^{-1} for both 2,6-dimethoxyphenol and 3,5-dimethoxy-4-hydroxybenzaldehyde and 21.9 mg L^{-1} for 4-methoxybenzyl alcohol. The large differences in the LOD can be explained by differences in the current–potential curves of the compounds.[24] Varying the detection potential might improve the LOD value. Furthermore, higher method standard deviations correspond to higher LOD values. The detection limits of glucose and sucrose using HPAEC-PAD amount to 6.67 and 2.61 mg L^{-1}, respectively, and are nearly two orders of magnitude lower than in a corresponding HPLC-RI method (Table 2).[25]

However, the detection limits using HPAEC-PAD are higher compared to those determined using a GC-MS method.[26] Using this method, detection limits of 0.15 and 0.28 mg L^{-1} for glucose and sucrose, respectively, can be obtained. Nevertheless, this method requires a derivatization step.[26] Depending on the type of calculation of the LOD, lower values than those specified here may be calculated for HPAEC-PAD methods (Table 2). Using HPAEC-PAD, the sugar degradation product 5-HMF can be quantified up to very low concentrations of 0.42 mg L^{-1} which are comparable to HPLC-UV (Table 2).[27,28] Therefore, due to the strongly inhibiting properties of 5-HMF in subsequent fermentation steps, the developed HPAEC-PAD method is better suitable than HPLC-UV for the detection of 5-HMF at low concentration and allows for the simultaneous carbohydrate quantification in the hydrolyzates. The detection limit of 1.64 mg L^{-1} for galacturonic acid using HPAEC-PAD found in this work corresponds to the one achieved earlier.[12] In contrast, the detection limits of ASL compounds determined using HPAEC-PAD in this study vary significantly, by more than two orders of magnitude between 0.14 and 21.9 mg L^{-1}, depending on the compound (Table 1). The same phenomenon of varying LOD between 0.05 and 1 mg L^{-1}, for different standards, however, was observed using HPLC-UV.[28] While several methods are capable of characterizing biomass hydrolyzates at least partially (Table 2), the key advantage of the HPAEC-PAD method presented here is its capability to characterize hydrolyzates with only one method while obtaining comparable LODs.

Characterization of biomass hydrolyzates

Since HPAEC-PAD allows for a simultaneous characterization of the liquid phase of a biomass hydrolyzate, it was used to analyze various biomasses for their composition based on the two-step acid hydrolysis (Fig. 3, 4 and S2†).[8]

Table 1 Analytical parameters of the model compounds found in biomass hydrolyzates

Compound	Natural polymer	Retention time [min]	Linearity, R^2 [—]	Sensitivity [(nC min)/(mg L^{-1})]	LOD [mg L^{-1}]	Method standard deviation [mg L^{-1}]
Rhamnose	Hemicellulose/pectin	6.500	0.9993	0.16	0.78	0.33
Arabinose	Hemicellulose	7.583	0.9991	0.23	1.49	0.63
Galactose	Hemicellulose	9.408	0.9992	0.30	0.97	0.41
Glucose	Cellulose/hemicellulose	10.383	0.9982	0.28	6.67	2.82
Mannose	Hemicellulose	11.167	0.9989	0.065	1.63	0.68
Xylose	Hemicellulose	11.758	0.9990	0.26	1.47	0.62
Fructose		15.525	0.9972	0.20	3.32	1.25
Sucrose		12.610	0.9982	0.31	2.61	0.76
Cellobiose	Cellulose	22.825	0.9962	0.18	0.26	0.11
Cellotriose	Cellulose	24.350	0.9983	0.37	0.44	0.17
Cellotetraose	Cellulose	25.417	0.9965	0.22	0.65	0.24
Cellopentaose	Cellulose	26.009	0.9948	0.18	0.38	0.14
Cellohexaose	Cellulose	26.200	0.9966	0.07	0.64	0.24
Galacturonic acid	Pectin	26.833	0.9994	0.084	1.64	0.61
Glucuronic acid	Pectin	27.067	0.9951	0.0023	2.58	0.96
5-HMF	Cellulose/hemicellulose	3.767	0.9978	0.20	0.42	0.18
2,3-Dimethoxybenzyl alcohol	Lignin	2.067	0.9989	0.20	0.35	0.15
3,4-Dimethoxybenzyl alcohol	Lignin	2.109	0.9913	0.0077	0.97	0.41
4-Methoxybenzyl alcohol	Lignin	2.705	0.9998	0.82	21.86	7.28
3,4-Dimethoxybenzaldehyde	Lignin	2.960	0.9989	0.20	9.23	3.49
1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol	Lignin	5.908	0.9940	0.15	4.23	1.60
2,6-Dimethoxyphenol	Lignin	27.600	0.9973	5.93	0.14	0.051
2-Methoxyphenol	Lignin	29.058	0.9978	0.021	1.62	0.60
4-Allyl-2-methoxyphenol	Lignin	36.942	0.9997	0.30	0.27	0.13
3,5-Dimethoxy-4-hydroxybenzaldehyde	Lignin	42.550	0.9955	8.36	0.14	0.05

Table 2 LOD values from the literature determined using diverse analytical methods

Substance	System	LOD	Reference
Glucose	GC/MS	0.15 mg L^{-1}	Medeiros and Simoneit 2007 (ref. 26)
	HPAEC-PAD	0.02 mg L^{-1}	Cataldi *et al.* 2000 (ref. 11)
	HPLC-RI	130 mg L^{-1}	Chávez-Serin *et al.* 2004 (ref. 25)
Sucrose	GC/MS	0.28 mg L^{-1}	Medeiros and Simoneit 2007 (ref. 26)
	HPLC-RI	160 mg L^{-1}	Chávez-Serin *et al.* 2004 (ref. 25)
5-HMF	HPLC-UV	0.3 mg L^{-1}	Albalá-Hurtado *et al.* 1997 (ref. 27)
	HPLC-UV	0.025 mg L^{-1}	Kermasha *et al.* 1995 (ref. 28)
Galacturonic acid	HPAEC-PAD	3.88 mg L^{-1}	Garna *et al.* 2004 (ref. 12)
Catechin	HPLC-UV	1 mg L^{-1}	Kermasha *et al.* 1995 (ref. 28)
Ferulic acid	HPLC-UV	0.1 mg L^{-1}	Kermasha *et al.* 1995 (ref. 28)
p-Coumaric acid	HPLC-UV	0.05 mg L^{-1}	Kermasha *et al.* 1995 (ref. 28)

Fig. 3, 4 and S2† show that the simultaneous determination of mono- and oligosaccharides as well as sugar degradation products, uronic acids and ASL compounds is possible. In particular for the characterization of orange, melon *etc.* peels, HPAEC-PAD should be preferred over the NREL/TP-510-42618 analytical procedure due to the capability to quantify pectin.[8] Fig. 4 incorporates the content of AIL, which is indeed not soluble and quantified gravimetrically to demonstrate the potential of the HPAEC-PAD method to obtain a nearly complete biomass composition analysis.

However, many biomass hydrolyzate samples still contain several unknown components, in particular barley, lime peel and spruce wood. Therefore, unknown peaks have to be identified using a structure elucidating system. Furthermore, the two-step acid hydrolysis procedure used has to be optimized for the complete quantification of all components of those biomasses which have a composition diverging significantly from that of wood. Further investigations shall also include the determination of ash, proteins, lipids *etc.* to close the mass balance.

The precision of the HPAEC-PAD method was investigated with a tenfold injection of a melon peel hydrolyzate based on the absolute and relative standard deviation (RSD) (Table S3†). The mono- and oligosaccharides, uronic acids, the sugar

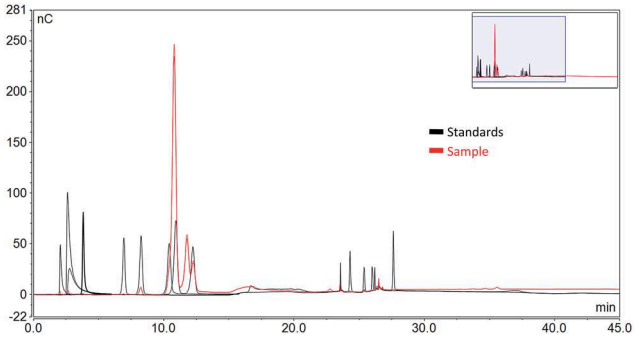

Fig. 3 Overlay of chromatograms from different standards and the spruce wood hydrolyzate. The spruce wood was hydrolyzed with a two-step acid hydrolysis. The obtained liquid was analyzed using the HPAEC-PAD method described here.

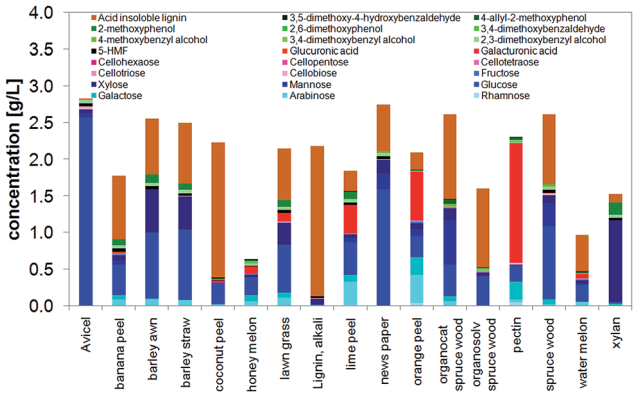

Fig. 4 Concentration of soluble compounds and acid insoluble lignin in the acid hydrolyzates of diverse biomasses. Concentration quantification of the specific soluble compounds was performed according to the developed HPAEC-PAD method. The theoretical maximum of concentration is 3 g biomass per L + the specifically added amount of water used for each polysaccharide hydrolysis step. For detailed information see Tables S1 and 2.†

degradation product and most of the lignin-based compounds (3,4-dimethoxybenzaldehyde and 3,5-dimethoxy-4-hydroxybenzaldehyde) have RSD values lower than 4% which indicate that the method is precise for these compounds. In contrast, one ASL compound, 4-allyl-2-methoxyphenol, has a higher RSD of 21.4%. This can be attributed to the very low concentration in the hydrolyzate of 0.28 mg L^{-1}. Quantification close to the LOD will always result in higher errors and higher RSD values.

Method comparison

Photometric methods are often used for the quantification of ASL in hydrolyzates.[8,29–32] HPAEC-PAD separates the ASL and carbohydrate compounds, motivating a comparison to the established sum parameters of ASL to the sum of lignin-derived compounds, respectively. Fig. 5 correlates the ASL content measured by the photometric method to the content measured using HPAEC-PAD for several model compounds (Avicel®, hemicellulose and lignin, alkali), grasses (lawn grass, barley

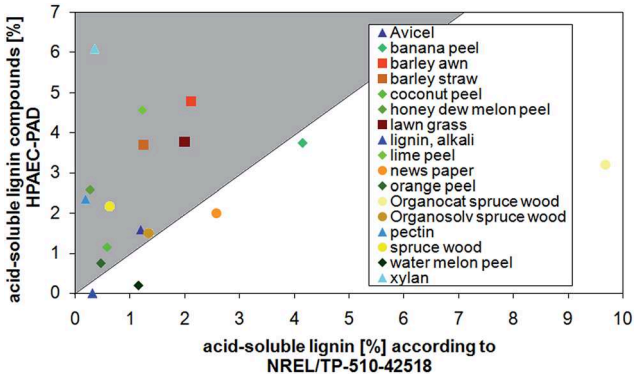

Fig. 5 Parity plot of the acid-soluble lignin compounds of biomass hydrolyzates comparing the HPAEC-PAD method with the acid soluble lignin content according to NREL/TP-510-42618. The sum of all measured acid-soluble lignin compounds with the HPAEC-PAD method for 17 hydrolyzates (wood, grasses, peels and commercially available standards) were correlated to the ASL sum parameter determined for the same hydrolyzates with the NREL method.

awn and straw), peels (banana, lime, melon, orange), newspaper and wood (native spruce as well as Organosolv- and Organocat-pretreated spruce).

It is striking that the substance classes form groups in the correlation. Generally, the photometric measurement without compound separation results in a sum parameter which is depending on the wavelength influenced by carbohydrate monomers, sugar degradation products and other soluble biomass derived compounds, thus, typically leading to an overestimation (Fig. 5).[29] Nevertheless, the sum of all ASL compounds using HPAEC-PAD may be too low due to unidentified and thus not assigned peaks in the chromatogram. Due to a retention time based quantification matrix compounds having the same retention time will lead to an overestimation using the HPAEC-PAD method.

Experimental

Chemicals and reagents

Sugar standards used for method development and external calibration were arabinose 99%, galactose > 99%, glucose > 99%, mannose 99%, rhamnose > 98% and xylose > 99%, all purchased from Dr Ehrenstorfer (Germany). Oligomeric saccharides (cellotriose > 95%, cellotetraose > 95%, cellopentaose > 95% and cellohexaose > 90%) were obtained from Megazyme (Ireland). The lignin model compounds (2-methoxyphenol ≥ 98%, 2,3-dimethoxybenzyl alcohol 99%, 2,6-dimethoxyphenol 99%, 3,4-dimethoxybenzyl alcohol 96%, 3,4-dimethoxybenzaldehyde ≥ 98%, 3,5-dimethoxy-4-hydroxybenzaldehyde ≥ 98%, 4-allyl-2-methoxy-phenol ≥ 98% and 4-methoxybenzyl alcohol 98%), the sugar degradation product (5-hydroxymethylfurfural > 99%, 5-HMF) and uronic acid standards (galacturonic acid ≥ 98% and glucuronic acid ≥ 98%) were all purchased from Sigma Aldrich (USA). The LMC 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol was kindly synthesized by Jacob Mottweiler (group of Prof. Bolm).[33]

Sulfuric acid 98% used for biomass hydrolysis was purchased from Roth (Germany). Equilibration and elution of the analytes were performed using 50% sodium hydroxide solution from Sigma Aldrich and sodium acetate from Thermo Scientific (USA).

Samples and sample preparation

Several potential biorefinery feedstocks were used for quantification experiments according to the NREL/TP-510-42618 procedure.[8] The fruits (banana, lime, honeydew- and watermelon as well as orange) whose peel was used for hydrolysis and the newspaper were bought in Aachen (Germany). Natural spruce wood grown in Germany was used as well as material pretreated by Organosolv without using a catalyst and Organocat procedures in a 300 mL high-pressure reaction system from Parr Instrument (USA).[34,35] Furthermore, lawn grass as well as barley straw and awn grown in Germany were used.

The acid hydrolyzate was obtained after a two-step acid hydrolysis of the respective biomass. This procedure started with a first hydrolysis step using 72 wt% sulfuric acid at a temperature of 30 °C for 60 minutes. The second hydrolysis step started immediately after diluting the hydrolyzate to 4 wt% sulfuric acid using DI water. This step was carried out in a 12 L-autoclave (Omnilab, Germany) at 121 °C for additional 60 minutes. After cooling down to room temperature, the AIL was separated from the liquid by filtration.

The liquid hydrolyzate was used for the quantification of cellulose and hemicellulose mono- as well as oligosaccharides, uronic acids and ASL compounds. Before being measured, the samples were filtrated (0.2 μm, PVDF, Roth, Ger) and diluted depending on the analysis method used (no dilution for the photometric measurement and a 1 : 10 dilution for the HPAEC-PAD method).

The solid residue which corresponds to the AIL was quantified after drying at 105 °C with a XA105 Dual Range Mettler Toledo (Switzerland) analytical balance according to the NREL/TP-510-42618 procedure.[8]

HPAEC-PAD instrumentation, software and method

The mono- and oligosaccharides, sugar degradation products as well as uronic acids and ASL compounds were separated using ion exchange chromatography (ICS-5000+, ThermoScientific), consisting of an AS-AP autosampler with sample temperature control (15 °C), a gradient pump and pulsed amperometric detection. It was equipped with a CarboPac™ PA100-column for separation (ThermoScientific) and an Au and an AgCl reference electrode for detection. The potential is set to 2.0 V after 0.4 s of equilibration with 0.1 V. After a constant ramp of 0.01 s the potential is set to 0.6 V. Additional 0.01 s later, the potential is set to −0.1 V for 0.06 s. Then the cycle starts again.

The flow was set to 1 mL min^{-1}. The column temperature (in the range of 30–50 °C) as well as the eluent composition (water, sodium hydroxide and sodium acetate) was experimentally optimized and is discussed in the results and discussion chapter. The final method uses an equilibration time of 15 min and a starting eluent of 85% water and 15% sodium hydroxide (100 mM). The resulting gradient is shown in Fig. 2.

The chromatograms were analyzed using the software Chromeleon 7.2 (ThermoScientific).

Photometric analysis of acid soluble lignin

The ASL content was calculated according to the NREL/TP-510-42618 procedure using a wavelength of 240 nm and absorptivity based on suggestions of Maekawa *et al.*[8,36] The photometric measurements were carried out using a Synergy MX photometer (BioTek, USA).

Validation of the HPAEC-PAD method

The standard compounds were injected separately in order to determine the retention time. The standards were used for calibration depending on their peak resolution. Peaks which were not completely separated were quantified as a sum parameter. The limits of detection (LOD) were calculated based on the calibration curve standard deviation according to Epshtein.[23] The precision of the injection was determined with a tenfold injection of the analytical standards. The relative standard deviation (RSD) was used for calculation of the precision.

Conclusions

A rapid HPAEC-PAD method for the quantification of acid-soluble cellulose, hemicellulose, pectin and lignin compounds as well as the sugar degradation product 5-HMF from biomass hydrolyzates in 70 min using only one device was successfully developed. The wide range of detectable compounds including mono- and oligosaccharides, 5-HMF, uronic acids and acid-soluble lignin compounds and in particular the competitively low detection limit for most of these compounds, particularly for 5-HMF, qualifies the HPAEC-PAD method for a fast screening of potential biomass substrates for a biorefinery process.

However, some unknown peaks in the acid hydrolyzates need to be identified with structure elucidating systems. This identification will allow for a better characterization as well as the optimization of the two-step acid hydrolysis for each substrate. To this end, HPAED-PAD may be the best choice to identify such an optimum because of its automatized measurement of all critical compounds which are essential for biomass hydrolysis including the degradation products. Additionally, the detailed composition of the differently pretreated biomass hydrolyzates can be used in the future *e.g.* for studies of lignin inhibition in fermentation processes.

Acknowledgements

This work was performed as part of the Cluster of Excellence "Tailor-Made Fuels from Biomass", which is funded by the Excellence Initiative of the German federal and state governments to promote science and research at German universities. The authors thank Jacob Mottweiler from the chair for Organic

Chemistry of the RWTH Aachen for providing the lignin model compound.

References

1 G. Aravantinos-Zafiris, V. Oreopoulou, C. Tzia and C. D. Thomopoulos, *LWT–Food Sci. Technol.*, 1994, **27**, 468–471.
2 W. J. J. Huijgen, J. H. Reith and H. den Uil, *Ind. Eng. Chem. Res.*, 2010, **49**, 10132–10140.
3 N. Park, H.-Y. Kim, B.-W. Koo, H. Yeo and I.-G. Choi, *Bioresour. Technol.*, 2010, **101**, 7046–7053.
4 B. Rivas, A. Torrado, P. Torre, A. Converti and J. M. Dominguez, *J. Agric. Food Chem.*, 2008, **56**, 2380–2387.
5 A. A. Shatalov and H. Pereira, *Ind. Crops Prod.*, 2013, **43**, 623–630.
6 X. Zhang, W. Qin, M. G. Paice and J. N. Saddler, *Bioresour. Technol.*, 2009, **100**, 5890–5897.
7 J. F. Saeman, J. L. Bubl and E. E. Harris, *Ind. Eng. Chem. Res.*, 1945, **17**, 35–37.
8 A. Sluiter, B. Hamas, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker, *Laboratory Analytical Procedures (LAP), Technical Report NREL/TP-510–42618*, 2008.
9 E. P. Crowell and B. B. Burnett, *Anal. Chem.*, 1967, **39**, 121–124.
10 S. Willför, A. Pranovich, T. Tamminen, J. Puls, C. Laine, A. Suurnäkki, B. Saake, K. Uotila, H. Simolin, J. Hemming and B. Holmbom, *Ind. Crops Prod.*, 2009, **29**, 571–580.
11 T. R. I. Cataldi, G. Margiotta, L. Iasi, B. Di Chio, C. Xiloyannis and S. A. Bufo, *Anal. Chem.*, 2000, **72**, 3902–3907.
12 H. Garna, N. Mabon, B. Wathelet and M. Paquot, *J. Agric. Food Chem.*, 2004, **52**, 4652–4659.
13 H. Garna, N. Mabon, K. Nott, B. Wathelet and M. Paquot, *Food Chem.*, 2006, **96**, 477–484.
14 V. Lebet, E. Arrigoni and R. Amadò, *Z. Lebensm.-Unters. -Forsch. A*, 1997, **205**, 257–261.
15 A. Uremovic, T. DokkGlawischnig, J. Schuseil, B. Saake, A. Borchmann, A. Herrmann and J. Puls, *Holz Roh- Werkst.*, 1994, **52**, 347–354.
16 A. Versari, S. Biesenbruch, D. Barbanti, P. J. Farnell and S. Galassi, *Food Chem.*, 1999, **66**, 257–261.
17 M. Pasta, F. La Mantia and Y. Cui, *Electrochim. Acta*, 2010, **55**, 5561–5568.
18 M. R. Wilkens, W. W. Widmer, K. Grohmann and R. G. Cameron, *Bioresour. Technol.*, 2007, **98**, 1596–1601.
19 M. Yoshida, Y. Liu, S. Uchida, K. Kawarada, Y. Ukagami, H. Ichinose, S. Kaneko and K. Fukuda, *Biosci., Biotechnol., Biochem.*, 2008, **72**, 805–810.
20 P. Bocchini, G. C. Galletti, S. Camarero and A. T. Martínez, *J. Chromatogr., A*, 1997, **773**, 227–232.
21 S. Camarero, D. Ibarra, M. J. Martínez and A. T. Martínez, *Appl. Environ. Microbiol.*, 2005, **71**, 1775–1784.
22 J. I. Hedges and J. R. Ertel, *Anal. Chem.*, 1982, **54**, 174–178.
23 N. A. Epshtein, *Pharm. Chem. J.*, 2004, **38**, 212–228.
24 C. M. Zook and W. R. LaCourse, *Curr. Sep.*, 1995, **14**, 48–52.
25 J. L. Chávez-Serin, A. I. Castellotte and M. C. López-Sabater, *J. Chromatogr., A*, 2004, **1043**, 211–215.
26 P. M. Medeiros and B. R. T. Simoneit, *J. Chromatogr., A*, 2007, **1141**, 271–278.
27 S. Albalá-Hurtado, M. T. Vecianna-Nogués, M. Izquierdo-Pulido and M. C. Vidal-Carou, *J. Agric. Food Chem.*, 1997, **45**, 2128–2133.
28 S. Kermasha, M. Goetghebeur, J. Dumont and R. Couture, *Food Res. Int.*, 1995, **28**, 245–252.
29 R. Hatfield and R. S. Fukushima, *Crop Sci.*, 2005, **45**(3), 832–839.
30 W. E. Kaar and D. L. Brink, *J. Wood Chem. Technol.*, 1991, **11**, 465–477.
31 P. Sannigrahi, D. H. Kim, S. Jung and A. Ragauskas, *Energy Environ. Sci.*, 2011, **4**, 1306–1310.
32 T. J. Schwartz and M. Lawoko, *BioResources*, 2010, **5**, 2337–2347.
33 J. Buendia, J. Mottweiler and C. Bolm, *Chem.–Eur. J.*, 2011, **17**, 13877–13882.
34 J. Wildschut, A. T. Smit, J. H. Reith and W. J. Huijgen, *Bioresour. Technol.*, 2013, **135**, 58–66.
35 T. vom Stein, P. M. Grande, H. Kayser, F. Sibilla, W. Leitner and P. D. de Maria, *Green Chem.*, 2011, **13**, 1772–1777.
36 E. Maekawa, T. Ichizawa and T. Koshijima, *J. Wood Chem. Technol.*, 1989, **9**, 549–567.