Comparison of ionization (ESI) of different biomass based anhydrosugars

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Introduction

As oil plays a significant role in the global economy, the rapidly growing consumption of fossil energy resources and the overall decline in oil reserves lead to the global energy crisis. As a result, many studies are based on the use of biomass as the most valuable renewable resource in the production of liquid fuels, materials and chemicals. Bio-oil is a complex mixture of compounds containing alkenes, aromatic compounds, phenols, furans, esters, aldehydes, ketones, sugars and acids. Carbohydrate derivatives, such as 1,6-Anhydro-beta-glucopyranose (or levoglucosan, or LG) and it's ketone - (1S,5R)-6,8-Dioxabicyclo[3.2.1]oct-2-en-4-one (or levoglucosenone, or LGO) are among the main products of thermochemical degradation of cellulose and are valuable platform chemicals. The LG and LGO are mainly determined using GC or HPLC. The UHPLC used in this study yields better results – higher resolution, and shorter analysis time. UV detection is used primarily for LGO assays because LGO and its by-products contain chromophore groups. But for LG analysis UV detector cannot be used because LG does not absorb in UV/VIS spectrum. This work focused on the use of mass spectrometry, to determine whether both anhydrosugars have similar ionization. In previous work the degradation of LGO in the presence of water was determined. The main LGO degradation products are furfural and hydroxylevoglucosenone (Figure 1). The chemical structure of hydroxylevoglucosenone is tentative.

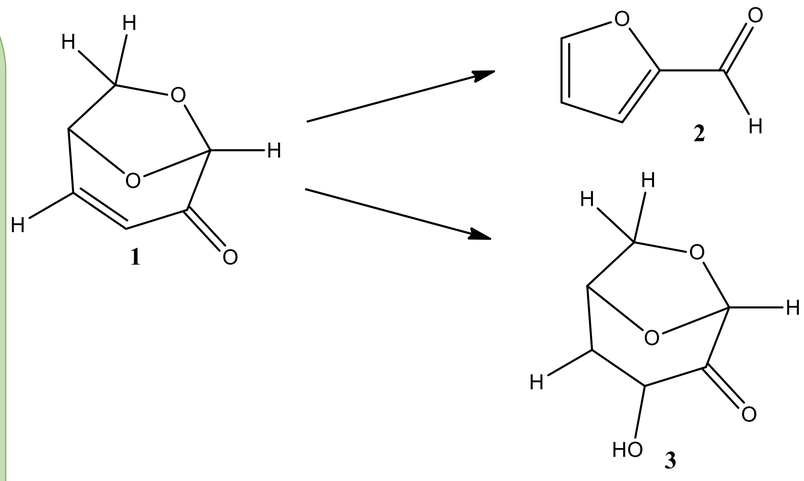

Fig. 1.. **Main LGO degradation products (1 – furfural, 2 – hydroxylevoglucosenone)**

Experimental

In this study we analyzed LG and LGO standards and also pyrolysis liquid samples, which were prepared, using lignocellulose obtained from hydrolyzed birch (*Betula pendula*) chips. Tested method parameters are shown in Fig.2. To determine the separation between LGO and its degradation products UV detector was used at 220 nm vawelenght. LGO derivatization was made with 2,4-dinitrophenylhidrazine (DNPH).

Analysed compound	Column	Mobile phase	Column temperature, °C	Ionization mode (ESI)
LGO, LGO-DNPH	CSH Phenyl-Hexyl (1.7 µm, 2.1 x 100 mm)	A – water (MilliQ) with 0.1 % FA; B – ACN Flow rate 0,4 mL·min^{-1} Gradient mode	30	+
LG	BEH Amide (1.7 µm, 2.1 × 100 mm)	A – water (MilliQ)/ACN (40/60) with 0,1 % NH_3 Flow rate 0,15 mL·min^{-1} Isocratic mode	60	-
LGO	CSH Fluoro-Phenyl (1.7 µm, 2.1 x 100 mm)	A – water (MilliQ)/ACN (40/60) with 0,1 % NH_3 Flow rate 0,15 mL·min^{-1} Isocratic mode	30	-

Fig.2. **LG and LGO determination method parameters**

Results and Discussion

Analysed compound	Column	Mobile fase additive	Ionization mode (ESI)	Separation (UV)	Ionization	Tested method parameters
LGO	CSH Phenyl-Hexyl	FA	+	• LGO and furfural are separated (k LGO= 0,98; k furfural = 1,15; α = 1,17). • LGO and hydroxylevoglucosenone are not separeted.	• In TIC ions with m/z 97 and 127 Da are determined. • Ion with m/z 97 Da corresponds to molecular ion of furfural $[Mfurfural-H]^+$.	
	CSH Fluoro-Phenyl	NH_3	-	• LGO and furfural are separated (k LGO = 2,16; k furfural = 2,38; α = 1,10). • LGO and hydroxylevoglucosenone are separeted (k = 1,30; α = 1,66).	• In TIC ions with m/z 95, 125 and 143 Da are determined. • Furfural does not ionizes. • Ions with m/z 95, 125 and 143 Da after the retention time of UV chromatogram corresponds to hydroxylevoglucosenone. • LGO does not ionizes.	• R^2 = 0,9993 (0,0013 – 0,2860 mg/mL) • LOD = 0,00010 mg/mL • LOQ = 0,0003mg/mL • Level 1 (80%): R = 94,5±0,5 mg/mL Level 2 (100%): R = 96,3±0,6 mg/mL Level 3 (120%) 3: R = 97,5±0,2 mg/mL • Accuracy (system): 0.3 % • Accuracy (method): 0.4 %
LGO-DNPH	CSH Phenyl-Hexyl	FA	-	• LGO-DNPH and its degradation product derivatives are separeted.	• m/z 305 Da = $[M(LGO-DNPH)-H]^-$ • m/z 275 Da = $[M(Furfural-DNPH)-H]^-$ • m/z 323 Da = $[M(Hidroksi-LGO-DNPH)-H]^-$	• R^2 = 0,991 (0,0027 – 0,0265 mg/mL) • R^2 = 0,994 (0,0530 – 0,3180 mg/mL) • LOD = 0,0007 mg/mL • LOQ = 0,002 mg/mL • Diluted 4x: R = 66 ± 3 mg/mL • Diluted 10x: R = 76 ± 3 mg/mL • Accuracy (system): 3 % • Accuracy (method): 4 %
LG	BEH Amide	NH_3	-		• m/z 161 Da = $[M(LG)-H]^-$ • m/z 323 Da = $[2M(LG)-H]^-$	• R^2 = 0,998 (0,0015 – 0,3005 mg/mL) • LOD = 0,00010 mg/mL • LOQ = 0,0003mg/mL • Level 1 (80%): R = 98,5±0,6 mg/mL • Level 2 (100%): R = 98,3±0,4 mg/mL • Level 3 (120%) 3: R = 97,9±0,5 mg/mL • Accuracy (system): 0.3 % • Accuracy (method): 0.4 %

Fig.3. **Obtained results fro LG and LGO determination methods with UHPLC-UV-QDa system**

Conclussions

According to the MS results, we can conclude that LGO does not ionizes without derivatization by utilizing ESI system, while its degradation products does. In comparison, biomass based anhydrosugar LG ionizes using ESI system in negative ionization mode. So, we can conclude that there is a significant difference in ionization between these two anhydrosugars. The differences of ionization can be explained by structural differences of these compounds – ketones poorly ionize in the ESI source. To obtaine ionization of LGO new method for LGO derivatization with 2,4-dinitrophenylhydrazine were made.

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