

## DETERMINATION OF CARBOHYDRATES IN LIGNOCELLULOSICS BY GAS CHROMATOGRAPHY OF ALDONITRILACETATES

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**SYNOPSIS.** The information of saccharides qualitative presence and quantitative abundance in lignocellulosics is very important from the viewpoint of the saccharides structure knowledge. In the paper the optimization is described of sugar aldonitrilacetates capillary gas chromatography method and its evaluation for the separation and quantification of carbohydrates in various samples. For the monosaccharides identification six aldonitrilacetates of standard carbohydrates (rhamnose, arabinose, xylose, manose, glucose, galactose) were prepared. Three sulphate spruce pulps were hydrolysed by SAEMAN et AL. (1954) method. Also holocellulose was prepared by WISE et AL. (1946) method from beech wood before and after prehydrolysis. Then the samples were derivatised to aldonitrilacetates. The gas chromatography was performed on Fisons 8310 DPFC GC with flame ionisation detector and DB-5 capillary column with inositol as an internal standard. The retention times, response factors, resolution and recovery of the standard carbohydrates aldonitrilacetates were received. The obtained results of the saccharides determination in the pulps and holocellulose showed the suitability of aldonitrilacetates for the saccharides from cellulosics analysis by method of the capillary gas chromatography.

**KEY WORDS:** carbohydrates, wood, pulp, gas chromatography, aldonitrilacetates

### INTRODUCTION

In lignocellulosics there is a big amount of saccharides (65-75%). The information about their qualitative presence and quantitative abundance is very important from the viewpoint of their structure knowledge. Saccharides and products of their conversion have a big importance in the course of controlling of the technological processes at the treatment of regenerative energy sources (WYMAN 2003, 2007, GUO et AL. 2008). Identification and quantification of monosaccharides are important also in the explanation of carbohydrate metabolism, e.g. in plants, soils etc.

There are various methods for the analysis of monosaccharides including high performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE) and gas chromatography (GC) (SMALLOW and LOW 1990, KAAR et AL. 1991, KAČÍK et AL. 1993, SUZUKI et AL. 1995, DAVIS 1998, DAHLMAN et AL. 2000, YE et AL. 2006, ZHANG et AL. 2007, ROVIO et AL. 2008).

In the HPLC analyses the elution times are shorter when compared to the same analyses by GC, when using HPLC there is no need to derivatise arising monosaccharides. However, gas chromatography is a very universal method to analyse carbohydrates in various samples, especially in lignocellulosic materials. Saccharides are thermally labile and nonvolatile hence they must be derivatised into volatile and stable compounds by using of GC, including alditol acetates, trimethylsilyl ethers, trifluoroacetates and *O*-isopropylidenes. Alditol acetates derivatisation incorporated in TAPPI T 249 cm-00 (2000) method is complicated, trimethylsilylation results in complex mixture due to formation  $\alpha$ - and  $\beta$ -anomers of the pyranoside and furanoside forms and these derivatives are unstable during storage LARRÉ-LARROUY and FELLER (1997). *O*-isopropylidenes yield one, or mainly one, product of common aldoses and derivatisation is simple (MORGENLIE 1975, MORGENLIE et AL. 1988, EKEBERG and MORGENLIE 2004), nevertheless the analysis is not widespread. Aldonitrilacetates can be prepared quantitatively in a short time, they are stable at the storage and each monosaccharide provides only one peak. Aldonitrilacetates are used in the analysis of saccharides in wood, pulps, holocellulose, cellulose, hydrolysates of various lignocellulosics, in soils etc. (SUZUKI et AL. 1995, DAVIS 1998, DAHLMAN et AL. 2000, LAUROVÁ and KUČEROVÁ 2005).

The aim of the present study was the optimization of sugars aldonitrilacetates capillary gas chromatography method and its evaluation for the separation and quantification of carbohydrates in various samples.

## EXPERIMENTAL

### Preparation of aldonitrile acetates of standard sugars

A mixture of six monosaccharides was dissolved in pyridine (0.05 cm<sup>3</sup> for 1 mg of sugar) and treated with hydroxylamine hydrochloride (1 mg for 1 mg of sugar) and heated in sealed glass ampoule at 90°C for 30 min. After cooling to room temperature acetic anhydride (0.15 cm<sup>3</sup> for 1 mg of sugar) was added and mixture was heated for another 30 min at 90°C. The cooled solution was evaporated to oven-dry state under vacuum at 60°C. The residue was dissolved in acetyl acetate and injected into the chromatograph.

### Hydrolysis of pulps

Three the sulphate pulps were hydrolysed by the method of SEAMAN et AL. (1954). In the first step of hydrolysis, 1 cm<sup>3</sup> of 72% H<sub>2</sub>SO<sub>4</sub> was added and the sample (100 mg) was kept in a water bath at 30°C for 1 hour. Hydrolysate was

diluted by adding of 28 cm<sup>3</sup> of distilled water and a secondary hydrolysis was performed at 120°C for 90 min. The hydrolysate was neutralized by addition of BaCO<sub>3</sub>, and then 5 cm<sup>3</sup> of distilled water, containing the internal standard (inositol) at 1 mg·cm<sup>-3</sup>, was added. Subsequently, the hydrolysate was evaporated to oven-dry state under vacuum at 40°C. The samples were derivatised to aldonitrile acetates as above.

## Gas chromatography analysis

The gas chromatography analysis was performed on Fisons 8310 DPFC GC equipped with flame ionization detector (FID) (Fisons Instruments). A DB-5 capillary column (DB-5, 30 m × 0.32 mm ID, film thickness 0.25 μm) was used to separate the aldonitrile acetates of the neutral sugars with a split ratio of 100:1. Helium was used as a carrier gas at a column flow rate of 1.7 cm<sup>3</sup>·min<sup>-1</sup>. The oven temperature was held 175°C for 4 min, and then it was increased at the rate of 4°C·min<sup>-1</sup> to 220°C. The temperatures of the injector and detector were 250 and 300°C, respectively. Inositol was used as an internal standard for sugars determination.

## RESULTS AND DISCUSSION

Figure 1 shows that aldonitrile acetates of six principal monosaccharides in wood products and also inositol (internal standard) were all separated with satisfactory values of resolution (Table 1). The FID chromatogram of each compound

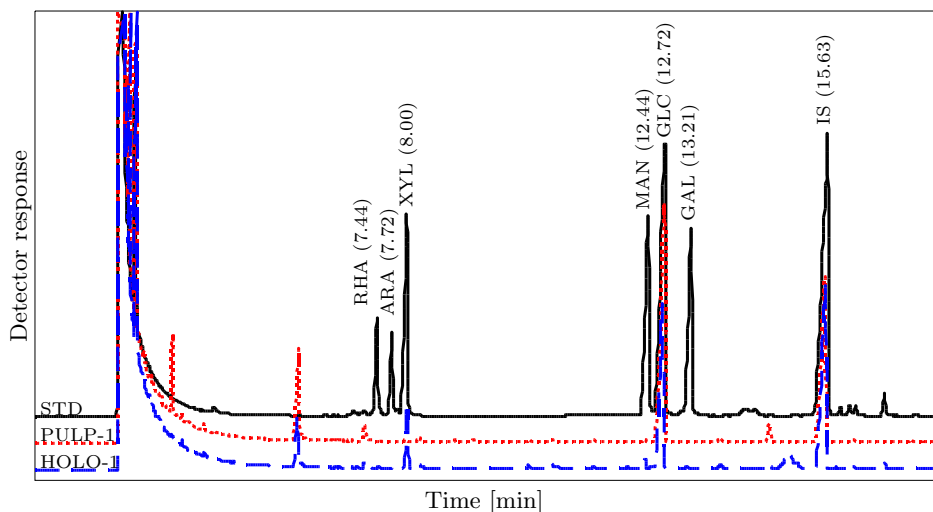


Fig. 1. Chromatograms of aldonitrile acetates of neutral sugars in standard mixture (STD), in pulp (PULP-1) and in the holocellulose (HOLO-1)

has a simple peak. This fact was confirmed by using gas chromatography-mass spectrometer (ZHANG et AL. 2007). Inositol is common internal standard used in carbohydrate analysis of lignocellulosics and it is included in TAPPI T 249 cm-00 method (2000), however also other internal standards for sugar determination were suggested (e.g. adonitol) (ZHANG et AL. 2007). YE et AL. (2006) applied external standard method with linear calibration curves in wide scale of sugars concentrations.

Table 1. Retention times ( $t_r$ ), response factors ( $f_x$ ), resolution between adjacent peaks ( $R_{i,j}$ ) and recovery of carbohydrates

Carbohydrate	$t_r$ [min]	$f_x$ (mean $\pm$ RSD [%], n = 5)	$R_{i,j}$	Recovery [%] (mean $\pm$ RSD [%], n = 5)
Rhamnose (RHA)	7.44	1.4184 $\pm$ 4.54	–	98.12 $\pm$ 4.52
Arabinose (ARA)	7.72	1.3958 $\pm$ 7.73	3.19	99.42 $\pm$ 5.01
Xylose (XYL)	8.00	1.3532 $\pm$ 6.84	2.85	101.21 $\pm$ 6.83
Manose (MAN)	12.44	1.2766 $\pm$ 4.36	34.86	97.86 $\pm$ 7.02
Glucose (GLC)	12.72	1.3487 $\pm$ 4.37	1.93	101.87 $\pm$ 5.29
Galactose (GAL)	13.21	1.4564 $\pm$ 6.24	3.27	102.26 $\pm$ 3.21
Inositol (IS)	15.63	1.00	13.89	–

The response factors for the sugars were obtained within a short range (Table 1) and this was important for quantitative determination of carbohydrates in various samples. The retention times, response factors, resolution and recovery of carbohydrates of the aldonitrile acetates are listed in Table 1. The recoveries of each neutral sugar relative to inositol were 100.12, what is in good agreement with ZHANG et AL. (2007).

In Table 2 there are the results of saccharides determination in three different sulphate spruce pulps. The yields are higher than 95%, which is in good accordance with the results of the neutral saccharides determination in pulps (SUZUKI et AL. 1995, DAVIS 1998, DAHLMAN 2000). The pulps content also a little amount of organic acids and residual lignin (Kappa numbers cca 2-6) beyond the neutral

Table 2. Amounts of carbohydrates ( $w\%$ ) in analysed samples

Carbohydrate	PULP 1	PULP 2	PULP 3	Holocellulose 1	Holocellulose 2
Rhamnose (RHA)	0.02	0.03	0.05	1.32	0.25
Arabinose (ARA)	0.16	0.15	0.11	1.24	0.91
Xylose (XYL)	4.27	4.90	4.17	24.03	15.57
Manose (MAN)	6.04	5.16	5.73	1.84	1.93
Glucose (GLC)	86.54	86.23	84.83	55.14	72.29
Galactose (GAL)	0.29	0.38	0.30	3.45	n.d.
Total	97.32	96.85	95.19	87.02	90.95

PULP 1, 2, 3 – sulphate spruce pulps from different enterprises.

Holocellulose 1 – holocellulose isolated from beech wood by WISE et AL. (1946).

Holocellulose 2 – holocellulose isolated from beech wood after the prehydrolysis (120°C, 16 h).

n.d. – not detected.

saccharides, which cause the total yields of saccharides are under 100%. In the beech holocellulose there is the total yield of monosaccharides 72.39%, it is caused by the presence of lignin and hemicelluloses with the high amount of uronic acids. At the beech wood prehydrolysis (120°C, 16 h) there are degraded the hemicelluloses with high amount of uronic acids and the saccharides yield increase (Table 2).

## CONCLUSIONS

From the obtained results we can see that aldonitrilacetates are suitable for the analysis of the carbohydrates in wood and wood materials. The monosaccharides provide only one peak and the capillary gas chromatography enables their separations with the enough resolution. The real samples analysis showed the described method is suitable also for the quantitative analysis of saccharides in the lignocellulosics.

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