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# ANALYSIS OF GLUCOSE OBTAINED FROM WOOD CARBOHYDRATES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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**ABSTRACT.** The aim of this study was the development and validation of a new method for glucose analysis obtained from woody biomass by steam-explosion and enzymatic hydrolysis. Quantification of glucose was made by liquid-liquid extraction, oximation and silylation and, finally, analysis by gas chromatography-mass spectrometry (GC-MS). Glucose derivatives obtained were identified by their GC retention time and the corresponding MS fragmentation. BSTFA was used as derivatization reagent to prepare the trimethylsilyl derivatives of glucose.

***Keywords****: glucose, wood carbohydrates, derivatization, GC-MS*

## INTRODUCTION

Ethanol production from woody biomass is a second generation biofuel process. It is necessary to find energy alternatives to petroleum due to the fossil fuel price and environmental requirements of the Kyoto protocol [1, 2]. The woody biomass is formed from cellulose, hemicellulose and lignin. Cellulose and hemicellulose are carbohydrates who can be converted into ethanol by saccharification and fermentation of glucose [3].

The bioconversion of woody feedstock to glucose contains three steps: pretreatment, hydrolysis, and glucose recovery/analysis. Cellulose is hydrolyzed to glucose, while hemicellulose is hydrolyzed to a mixture of pentoses and hexoses (glucose, mannose, galactose, xylose and arabinose). Cellulose can be chemically or enzymatically converted to glucose [4, 5].

The most used analytical techniques for glucose quantification are high-performance liquid chromatography (HPLC), gel permeation, thin layer, ion exchange and gas chromatography (GC). Derivatization is needed to produce

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more volatile compounds for gas chromatographic analyses of glucose [6, 7]. This technique was used for sugars quantification from food and soil. Usually, identification of sugar from lignocellulosic material was done by HPLC. The resolution of HPLC is not always sufficient to separate all mono and oligosaccharide of inters, and also quantification is limited. Gas chromatography coupled with mass spectrometry has the advantage of rapid identification of unknown compounds [8, 9].

The purpose of this paper is the development of a new analysis method based on GC-MS for quantification of glucose obtained by enzymatic hydrolysis of cellulose from wood. The method employed for glucose quantification is liquid-liquid extraction, followed by oximation and silylation with BSTFA.

## RESULTS AND DISCUSSION

Glucose was obtained from woody biomass by steam-explosion pretreatment and enzymatic hydrolysis of cellulose. Wood is a renewable source of glucose [3]. Cellulose separation of hemicellulose requires a hard pretreatment. Steam-explosion is a physico-chemical pretreatment, used for cellulose separation.

The solid fraction obtained after steam explosion pretreatment was enzymatically hydrolyzed using cellulase enzymes complex. The solid material was assumed to consist of lignin and cellulose only.

The glucose concentration was determined by two steps derivatization procedure: oximation and silylation, followed by GC-MS determination. Fig.1 shows the chromatogram of glucose using hydroxylamine hydrochloride in pyridine and BSTFA obtained by GC-MS.

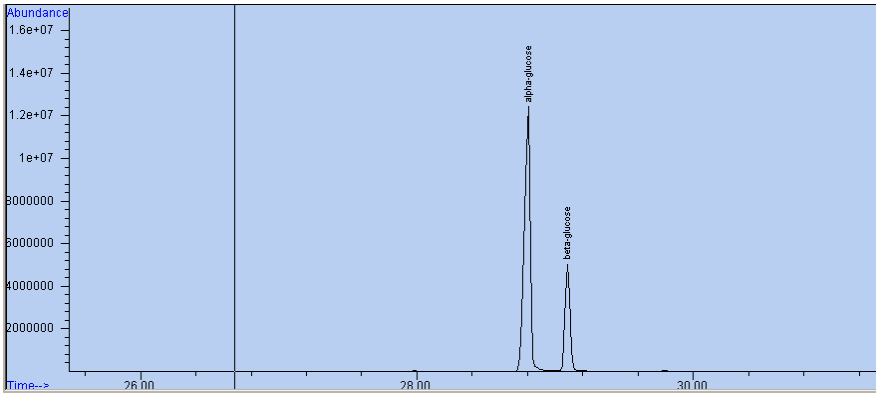


**Figure 1.** The SIM chromatogram of the glucose after oximation and silylation with BSTFA

Glucose contains six hydroxyl-groups thus the hexa-TMSi derivatives are formed. In SIM chromatogram, hexa-TMSi derivatives are evidenced by the presence of the two GC peaks due to the both 1α- and 1ß- compounds. A good chromatographic separation was obtained with a resolution better than 1.5 for 1α- and 1ß- glucose.

The mass spectra of hexasilylated derivatives are mainly characterized by the *m/z* 319, 205 and 147, respectively. Mass [$(CH_3)_3Si$–, *m/z*, 73] was obtained that corresponds to the mono-$TMS^+$ ion.

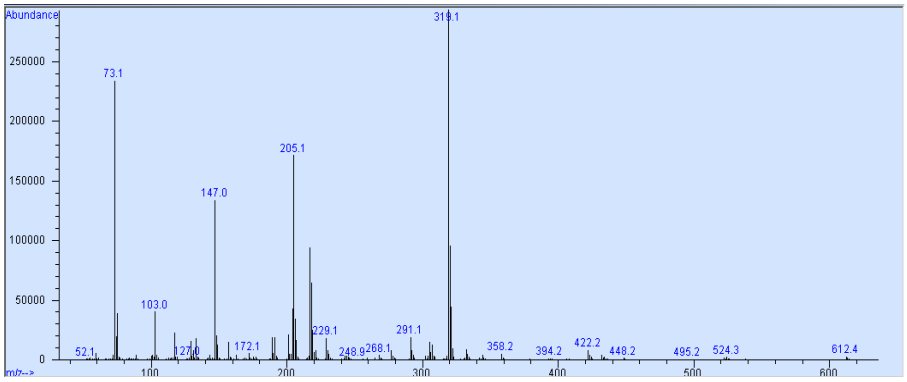


**Figure 2.** The MS spectra of the ions for the quantitative and qualitative analysis of the silylated derivatives of glucose

Retention time is the parameter used for correct identification of the glucose isomers obtained by extraction and derivatization of the enzymatic hydrolysates. The peaks that corresponded to the 1α- and 1ß-glucose appear at the retention time 28.709 and 29.004 min.

For glucose quantification, external calibration was used. Standard solutions of glucose were prepared at five different concentrations (1 mg/ml, 3mg/ml, 5mg/ml, 7mg/ml and 10mg/ml), derivatised and analyzed by GC-MS. The limit of detection (LOD) for glucose was calculated as the concentration that corresponds to the three times the standard deviation of the blanks (3s criterion, 10 independent blanks for each analyte) [8]. The limit of detection was calculated as being 0.222 µg for 1α-glucose and 0.171 µg for 1ß-glucose, respectively.

The chromatogram of the glucose resulted from enzymatic hydrolysates after oximation and silylation derivatization process is shown in Fig. 3.

The recovery of glucose from samples was evaluated by using a glucose solution. Six experiments were done in parallel. Glucose was extracted, filtrated and concentrated as presented at experimental section. 1 mg extract was dissolved in 1 ml hydroxylamine hydrochloride solution and derivatized, followed by GC-MS analysis. The calculated degree of recovery was $91 \pm 5.2$ %.

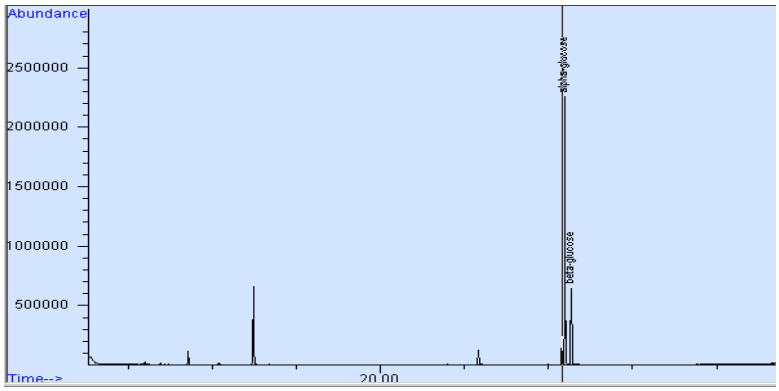


**Figure 3.** The chromatogram of the glucose-TMS oxime obtained from enzymatic hydrolysates

The relative standard deviation (RSD) of the peak area of the glucose derivatives in the chromatogram (calculated for 6 replicates of a solution containing glucose derivates) was less than 1%.

## CONCLUSIONS

The analytical results show that oxime-TMS derivatization of glucose is a suitable method for GC-MS identification. The products of derivatization can be easy identified by combination of their retention time and mass spectra. Derivatization of carbohydrates allows the quantitative determination of glucose in different cellulose and hemicellulose fractions. Glucose obtained from wood can be easily fermented to bioethanol, a renewable fuel that can replace gasoline.

Extraction of glucose from enzymatic hydrolysates, oximation and silylation with BSTFA is a simple and fast analytical method.

Both recovery and LOD values obtained for glucose using the method described here is comparable with those obtained by other authors [7].

## EXPERIMENTAL SECTION

Glucose was obtained from wood by steam-explosion pretreatment and enzymatic hydrolysis of cellulose according to Sassner method with modifications [10].

Steam-explosion involves separating the cellulose as a solid fraction at high temperature and pressure. The pretreated material was separated by filtration in two fractions: a solid material and a liquid phase. The solid material (cellulose and lignin) is enzymatically hydrolyzed to glucose. The glucose content was determined by GC-MS.

The optimal parameters of the derivatization method used in this study (temperature, derivatization time, extraction time) were selected according to other studies [7, 8].

### *Analytical methods*

Glucose obtained after enzymatic hydrolysis was extracted from the solution in 30 ml mixture of dichloromethane:methanol (2:1 v/v). The extract was concentrated by rotary evaporator; total extract was dried using a stream of filtered nitrogen gas. 1 mg extract or standard glucose was dissolved in 1 ml hydroxylamine hydrochloride solution (2.5%), heated to 80℃ for 30 min. After oximation, the derivatives were silylated by adding 300 µL BSTFA and the reaction mixture was heated at 80℃ for 10 min. One microliter of silylate derivative was injected to the gas chromatograph [7, 8].

### *Chemicals*

All chemicals were of analytical reagent grade. Glucose, pyridine, hydroxylamine hydrochloride ($NH_2OH.HCl$), dichloromethane, methanol were purchased from Merck (Darmstadt, Germany). The derivatization agents N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were purchased from Sigma–Aldrich.

### *Instrumentation*

A gas chromatograph 6890N (Agilent Technologies) coupled with a mass spectrometer 5973N MSD (Agilent Technologies) and a capillary column HP-5 MS (30 m×0.25 mm×0.25 µm) were used to analyze the glucose concentration.

### *GC-MS analysis*

For quantitative determination of glucose, the MS system was operated in SIM mode. The carrier gas was helium at constant flow rate of 1.0 $mLmin^{-1}$. The GC column temperature program applied was as following: the initial oven temperature was set at 65 ℃, held for 2 min, temperature increase of 6 $℃.min^{-1}$ to 300 ℃, followed by the isothermal hold at 300° C for 15 min.

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