Development of a GC-MS Method for 5-hydroxymethylfurfural Determination in Wood after Steam-explosion Pretreatment

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The aim of this study was the development and validation of a new gas chromatography – mass spectrometry (GC-MS) method used for the analysis of 5-hydroxymethylfurfural (HMF) in water soluble wood, after steam-explosion pretreatment. Two different methods were used for HMF extraction from water soluble wood, before gas chromatographic analysis: liquid-liquid extraction (LLE) with dichloromethane and solid phase extraction (SPE) method, and the analytical parameters of the two methods were compared. The recoveries of HMF in spiked were found to be in the range of 76-85% for LLE and 85-95% for SPE.

Keywords: wood analysis, 5-hydroxymethylfurfural, GC-MS, BSTFA, solid phase extraction

Woody biomass is the most abundant renewable resource and can be used for alternative energy sources and chemicals [1-4]. The major components of wood are: cellulose, hemicellulose and lignin [5-8]. Cellulose and hemicellulose could be chemical [9-10], biochemical [11], physic [12] and physico-chemical [13-15] depolymerized to sugars.

Most of the chemical transformation of wood into components was performed using solvents, requires long processing time and presence of chemicals [16]. Hexoses present in hemicellulosic phase are degraded to 5-hydroxymethylfurfural (HMF) during pretreatment for separation of wood into components [17-19]. HMF is very toxic furan derivatives found in all sugars: in acid pretreated wood hydrolysates and also in some foods [20, 21].

The existing methods for HMF analyses are classical methods as colorimetry and spectrophotometry, and also advanced techniques, such as high-performance liquid chromatography (HPLC) with UV detection and gas chromatography (GC). Generally, to increase the sensitivity and to improve the chromatographic separation is used a derivatization procedure. Several publications reported presence of HMF in food, in urine, wine by GC-MS [22-25]. The methods used for extraction of HMF followed by GC-MS are: liquid-liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction (SPME) with and without derivatization, depending of the raw material [26, 27].

In recent year, woody biomass was in attention as a renewable resource of energy, in particular for bioethanol production. One of the important stages of this process is the pretreatment process for cellulose and hemicellulose separation and for this are used several techniques [28]. Steam-explosion pretreatment can be used for hydrolysis of wood in presence of water, for cellulose, hemicellulose and lignin separation [29-31]. Water is used in this case as green medium for separation of water soluble products from wood based on changes in the dielectric properties of water at high temperature and pressure when approach those of organic solvents [32].

Impregnation with sulphuric acid of woody biomass previous to steam-explosion pretreatment improves the recovery of hemicellulosic sugars and secondary products in liquid phase [33, 34]. Presences of H_2SO_4 accelerate conversion of hexoses into HMF. HMF can be recovered from water soluble wood and can be used as a raw material for the synthesis of precursors of pharmaceuticals, thermoresistant polymers, and macrocyclic compounds, and particularly for the synthesis of dialdehydes, ethers, amino alcohols, and other organic intermediates (solvents, surface active agents, phytosanitary products, and resins) [35, 36].

The purpose of this paper is to compare two different extraction methods for extraction and analysis of HMF in wood: liquid-liquid extraction and solid phase extraction both followed by silylation using N,O-*bis*(trimethylsilyl) trifluoroacetamide (BSTFA). The derivatized compounds were determined by GC-MS, method that has not been applied before for this purpose.

Experimental part

Chemicals and reagents

The fir wood (*Abies alba* Mill.) species were collected locally and used as raw material. The dried material was stored in plastic bags at room temperature. All chemicals were analytical reagent grade. 5-hydroxymethylfurfural was purchased from Sigma-Aldrich (St. Louis, MO, USA). Pyridine, dichloromethane, methanol, acetic acid, sulphuric acid and sodium sulphate were purchased from Merck (Darmstad, Germany). BSTFA, hexamethyldisilazane (HMDS), HMDS + TMCS + Pyridine, 3:1:9 (Sylon HTP) were obtained from Supelco (Bellefonte, PA, USA).

Instrumentation

A GC-MS system containing a gas chromatograph (Agilent technologies, 6890N GC) coupled with mass spectrometer (Agilent technologies, 5973N MSD) and capillary column of HP-5 MS (30m length x 0.25mm) I.D. x 0.25 μm HP-3 MS film thickness was used. SPE extractions were performed on the Visiprep DL Vacuum Manifold for 12 samples from Supelco Inc. (Bellefonte, PA, USA). For solid-phase extraction SPE cartridges, three cartridges were used: Strata-X (200 mg, 6 mL) cartridges were purchased from Phenomenex (Torrace, CA, USA), Lichrolut RP-18 (40-63 μm) cartridges were purchased from Merck (Darmstad, Germany) and Oasis HLB (200 mg, 6 mL) cartridges were

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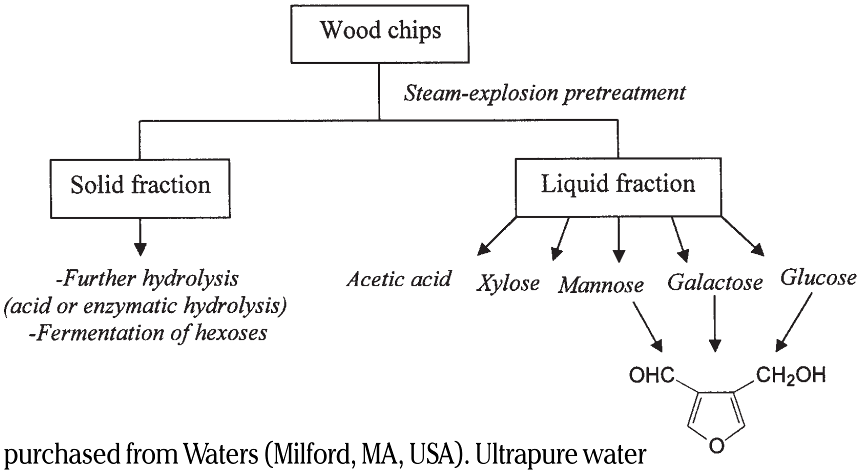

Fig. 1. Overview of experimental scheme

purchased from Waters (Milford, MA, USA). Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). For the SPME extraction a manual fiber holder Supelco Inc. (Bellefonte, PA, USA) with a 50μm of DVB/CAR/PDMS fiber Supelco Inc. (Bellefonte, PA, USA) were used. The *p*H was determined using a pH meter Jenway 3340). Extracts were dried on a Laborota 4010 (Heidolph, Germany) rotavapor using a Ilmvac Vacuum pump (Germany).

Wood pretreatment

A reactor (Parr Instruments, Illinois, USA) having 1L capacity was used for pretreatment method of wood. An amount of 25 g wood was weighed, and then 175 mL water containing sulfuric acid as catalyst (*p*H 2) was added. The pretreatment was performed at three different temperatures: 180, 190 and 200°C for a residence time of 10 min in all cases. The pretreated material was separated in solid fraction and liquid fraction containing the degradation products. The solid fraction was washed with water, and liquid fraction was divided into precipitate (lignin insoluble) and soluble fraction by the centrifuge and those would be used to characterize by-products (fig.1).

Liquid-liquid extraction procedure

A stock solution of HMF (1000 μg mL^{-1}) was prepared in pyridine. Calibration standard solutions with concentrations in the range of 50-250 μg mL^{-1} were prepared by diluting the stock solution with pyridine. 1 mL from each solution was silylated by adding 300 μL BSTFA and the reaction mixture was heated at 60°C for 10 min.

Liquid fraction obtained after steam-explosion was extracted from the solution in 15 mL dichloromethane. The extract was concentrated by rotary evaporator; total extract was dried using a stream of filtered nitrogen gas. The sample was then derivatized in the manner as the reference compound. All standard mixture solution was stored at 4°C. All analyses were carried out in triplicate.

SPE extraction procedure

Prior to extractions the cartridges were treated with 5 mL methanol, and 1mL of acetic acid aqueous solution 0.1% (v/v). Aqueous samples (1 mL) were passed through cartridges, followed by 1 mL water. Cartridges were dried by vacuum, and elution was carried out with 2 mL methanol. The effluent was evaporated to dryness using the rotary evaporator at 40°C and a steam of nitrogen. The extract was diluted with 1 mL pyridine and was silylated by adding 300 μL BSTFA and keeping for 10 min at 60°C.

GC-MS analysis

The carrier gas was helium with a constant flow rate of 1 mL min^{-1}. The injector temperature was 250°C, and the splitless injection mode was used. Samples were analyzed in EI full-scan data acquisition over the range *m/z* of 50-300. The temperature program was the following: initial oven temperature set at 70°C, held for 2 min, followed by an increasing of temperature with a rate of 12°C min^{-1} to 300°C, held for 2 min.

Results and discussion

HMF is produced from degradation of hexoses (glucose, mannose and galactose) present in wood. Generally, hydrolysis of hemicelluloses from wood was done using acids, water, organic solvents or alkaline agents. In this study, steam-explosion pretreatment of wood was applied for HMF formation. Using this type of wood pretreatment, hemicelluloses are recovered in monomeric forms in liquid fraction. This pretreatment performs two functions: the function of the pretreatment of wood decomposition and direct hydrolysis of hemicellulose to monomers sugars and HMF.

Three temperatures of reactor were used: 180, 190 and 200°C and time was set 10 min in all cases. The amounts of HMF obtained from wood at the three temperatures used for decomposition were compared.

The analysis of HMF was done by two extraction method, liquid-liquid extraction and SPE extraction. Analysis of HMF from wood is influenced by more factors: derivatizing agent, extraction procedure, temperature, time, etc. [26].

Optimization of the derivatization procedure

Liquid-liquid extraction and SPME extraction without derivatization were tested for HMF extraction from wood, according to Gaspar studies [26]. The silica fiber used for SPME extraction was DVB/CAR/PDMS. This extraction method could not be applied for the extraction of HMF from wood.

HMF contains a hydroxyl labile group that can be silylated with a derivatization agent. On this basis, we used derivatization method for the volatilization of the HMF for subsequent analysis by GC-MS. HMDS and pyridine were used for sylilation, according Molnar-Peer method [27]. However, using this method the recovery was very low. The derivatizing agent HMDS+TMCS+Pyridine (3:1:9) was also tested for sylilation of the extract, but no satisfactory results were obtained. Due to the fact that HMF is very soluble in water, the first solution was prepared by dissolving HMF solid in water, followed by extraction and finally derivatization with HMDS+TMCS+Pyridine (3:1:9) at 60°C for 10 min according to for HMF extraction from urine, but this procedure does not give any results.

Pyridine was chosen as solvent because is a polar solvent and favours the TMS reaction. BSTFA was found to be the best reagent for HMF derivatization using pyridine as solvent.

The temperature is a factor that affects the stability of HMF. Temperature of 60°C for 10 minutes was chosen according to Jöbstl D. [22], but using BSTFA as derivatization reagent.

Optimisation of the extraction procedure

Liquid phase resulted after steam-explosion reaction of wood was used for HMF analyses. Because matrices of liquid fraction are very complex, we have to found the best extraction and clean-up procedure prior to derivatization method. Liquid-liquid extraction was used, using dichloromethane and ethyl acetate according to Teixidó method for extraction of HMF from foods [31]. The best results were obtained using only dichloromethane as extraction solvent. 15 mL of solvent was used in all cases.

SPE was used to reduce volume of solvent, volume of sample and the time of extraction. Solid phase extraction was selected according to Beranek method [37]. For SPE extraction, three cartridges: Strata-X (200 mg, 6 mL), Lichrolut RP-18 and Oasis HLB (200 mg, 3 mL) were tested. Extraction procedure for each of them is presented in section SPE extraction procedure. All the cartridges were preconditioned with methanol and acetic acid solution.

Recovery studies were carried out by spiking samples (aqueous samples of liquid phase resulted after steam-explosion pretreatment) with amount of HMF (solution of HMF was prepared in water). Oasis HLB cartridges gives the best recoveries (90%) and Lichrolut RP-18 cartridges give the lowest recoveries (40%). Oasis HLB cartridges were have been chosen for the extraction of HMF from liquid phase resulted after steam-explosion pretreatment.

Chromatography of HMF standard solution

Figure 2 shows chromatogram of 5-hydroxymethyl-furfural standard solution. The retention time of HMF was 9.322 min.

HMF contains one hydroxyl-group, thus one-TMSi derivate is formed. The mono derivate for HMF was evidenced by the presence of *m/z* 183, 169, 109, indicating silylation of hydroxyl-group. The *m/z* 183 corresponding to the ion $[M-CH_3]^+$ was selected for quantification and molecular ion at 169 corresponding to $[M-COH]^+$ and 109 to the $[M-OTMS]^+$.

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

The linearity was studied using standard solution of HMF in concentration of 50, 100, 150, 200, 250 $\mu g\ mL^{-1}$ in pyridine. A good linearity was obtained with a correlation coefficient of 0.997.

Recovery studies were carried out by spiking samples (aqueous samples of liquid phase resulted after steam-explosion pretreatment) with HMF. Six experiments were done in parallel. The SPE extraction methods show the best recovery (average recovery were 85.5 – 95.4%) comparative with LLE extraction method (average recovery were 75.5 – 85.9%).

Limit of detection (LOD) for HMF was calculated as lowest concentration that can be determined with an acceptable level of repeatability and fidelity, by consecutive dilutions. The limit of detection was calculated as being 3 $\mu g\ mL^{-1}$.

The limit of quantification (LOQ) for HMF was calculated to the three times of limits of detection. LOQ for HMF was calculated as being 9 $\mu g\ mL^{-1}$. The method repeatability

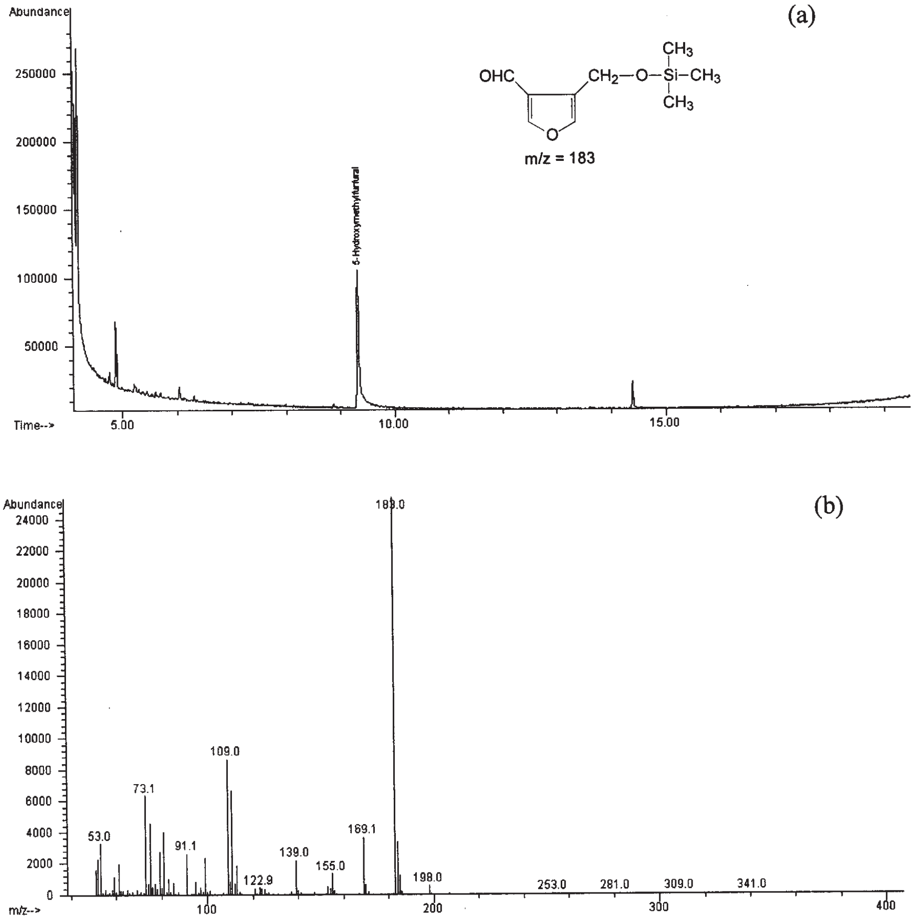

Fig. 2. GC-MS data for HMF standard solution: (a) GC-MS chromatogram for a derivatizated solution of HMF; (b) mass spectrum of TMSi-HMF

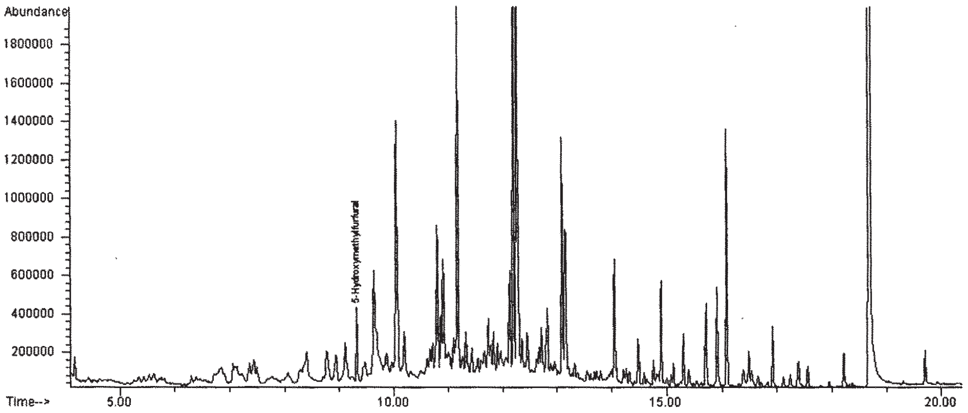

Fig. 3. The gas chromatogram of the TMSi-HMF compounds obtained by LLE-derivatization method

varied between 6 and 8% for LLE method, and 4 and 6% for SPE method. Repeatability was studied using the same cartridges, in six replicate.

Comparison of SPE and LLE for extraction of HMF from wood

Samples were analyzed by both extraction procedures. The GC chromatogram of the HMF obtained after derivatization of the liquid fraction resulted after steam-explosion pretreatment using LLE extraction and is shown in figure 3. The GC chromatogram of the HMF obtained after derivatization of the liquid fraction resulted after steam-explosion pretreatment using SPE extraction and is show in figure 4.

In table 1 is shown the comparison between SPE and LLE.

SPE extraction method has many advantages comparative with LLE methods: better recoveries, lower time-consumption, lower volumes of sample and reagents, being in this way environmental-friendly.

Steam-explosion pretreatment carried out in a lab scale apparatus (1L steam Parr reactor) to ensure a good process control and effective recovery of pretreatment fractions. The pretreatments were performing at three temperatures: 180, 190 and 200°C for a residence time of 10 min in all cases.

In figure 5 it was evaluated the concentration of the HMF depending on temperature.

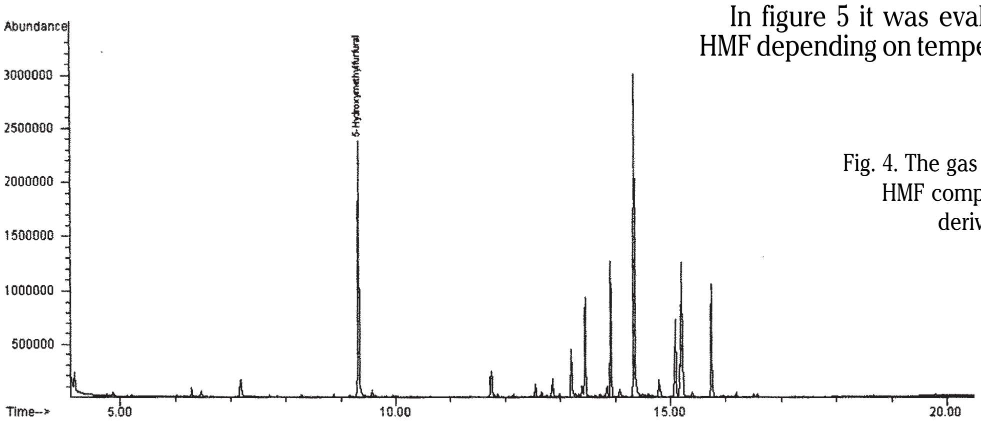

Fig. 4. The gas chromatogram of the TMSi-HMF compounds obtained by SPE derivatization method

Paremeters	LLE	SPE
Recovery (%)	75.8 – 85.9	85.5 – 95.4
Volume of samples	10 mL	1 mL
Volume of solvents	15 mL	7 mL
Time (min)	45	20
Performing	Complex	Simple
To environment	More polluting	Less polluting

Table 1
COMPARISON BETWEEN LLE AND SPE

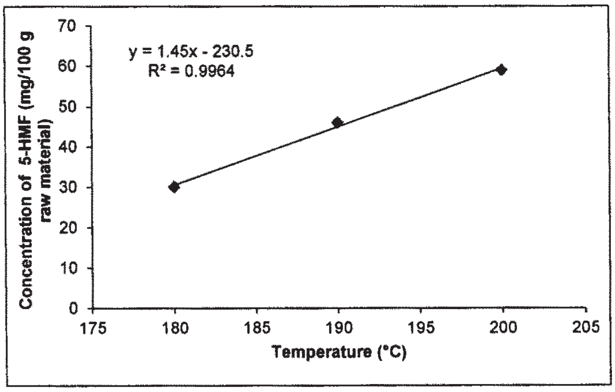

Fig. 5. Variation of HMF concentration depending on temperature

Figure 5 shows concentration obtained after steam-explosion pretreatment depending on temperature, concentration of HMF has a linear distribution function of temperature.

Conclusions

The liquid-liquid extraction and SPE extraction of HMF from wood were applied. SPE extraction was demonstrated to be the advantageous comparative with LLE extraction. The method is simple, rapid and gives a good recovery. Steam-explosion pretreatments were applied for HMF formation. The results show that temperature has a large influence on the formation of HMF, the increasing of temperature in steam-explosion pretreatment produces increasing amounts of HMF.

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