Tampereen ammattikorkeakoulu

Determination of Furfural from Wastewater with Headspace GC/MS

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LAINE, HEINI:
Furfuraalin määritys jätevedestä Headspace-GC/MS-tekniikalla
Opinnäytetyö 38 sivua, joista liitteitä 4 sivua
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Opinnäytetyön kokeellinen osuus tehtiin kesällä 2022 Tampereen yliopiston tekniikan ja luonnontieteiden tiedekunnassa, materiaalitieteen ja ympäristötekniikan yksikössä. Opinnäytetyön tavoitteena oli menetelmäkehitys furfuraalin määritykseen jätevesinäytteistä HS-GC/MS-laitteistolla. Opinnäytetyön tarkoituksena oli erilaisten koejärjestelyjen avulla testata laiteparametreja, kehittää tulosten sekä kirjallisuuden pohjalta menetelmä ja määrittää sen avulla jätevesinäytteiden furfuraalipitoisuus.

Jätevesinäytteet on saatu biojalostamolta, ja niiden tarkempi alkuperä ei salassapitosyistä ole tiedossa. Näytteiden analysointi on osa suurempaa projektia. Päämäärä menetelmäkehityksen taustalla oli edistää furfuraalin talteenottoa. Furfuraalilla on monia teollisia käyttötarkoituksia, joista ajankohtaisin on jatkokäsittely biopolttoaineina käytettäviksi yhdisteiksi.

Tavoitteeseen ei täysin päästy, koska projektin aikana ilmenneet laiteogelmat vaikeuttivat työskentelyä. Furfuraalin määritykseen saatiin kuitenkin kehitettyä toimiva menetelmä, jota käytettiin jätevesinäytteiden analysointiin. Näytteiden furfuraalipitoisuudet olivat odotettua matalammat, joten menetelmän jatkokehitys vaatisi ainakin uuden standardisuoran tekemisen ja mahdollisesti muuta lisätyötä, kuten ulossuolaustekniikan testaamista menetelmän hiomiseksi.

Asiasanat: furfuraali, jätevesianalyysi, menetelmäkehitys, headspace, GC-MS

ABSTRACT

Tampere University of Applied Sciences
Degree Programme in Laboratory Engineering

LAINE, HEINI:
Determination of Furfural from Wastewater with Headspace GC/MS

Bachelor's thesis 38 pages, appendices 4 pages
November 2022

Experimental part of the Bachelor ‘s thesis was conducted in summer 2022 at Tampere University’s Faculty of Engineering and Natural Sciences, in Materials Science and Environmental Engineering unit. The objective of the thesis was to develop a method for determination of furfural in wastewater samples using HS-GC/MS equipment. The purpose of the thesis was to test device parameters with different test setups, to develop a method based on the results and a literature review, and use the method to determine the furfural content of wastewater samples.

Wastewater samples were obtained from a biorefinery, and their exact origin was not disclosed due to confidentiality reasons. Analysis of the samples is part of a larger project. The goal behind the method development was to promote the recovery of furfural. Furfural can be utilized in several different industries, and lately has been researched as a platform chemical for biofuels.

The objective was not fully realised because of equipment problems that occurred during the project. However, a functional method with sufficient furfural detection was developed, and it was applied for analysis of wastewater samples. The furfural concentrations of the samples were lower than expected, so further development of the method would require making a new calibration curve and possibly other additional work, such as testing of salting-out technique.

Key words: furfural, wastewater analysis, method development, headspace, GC-MS

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ABBREVIATIONS

FID	Flame ionization detection
GC	Gas chromatograph
HPLC	High-performance liquid chromatography
HS	Headspace
MS	Mass spectrometer
NIST	National Institute of Standards and Technology
SIM	Selected ion monitoring

1 INTRODUCTION

There is an increasing demand for renewable alternatives for fossil fuels because of global warming. Lignocellulosic biomass as a source of biofuels has been researched in the recent years as an environmentally friendly alternative for fossil fuels. Lignocellulosic biomass refers to leftover plant material, such as forestry or agricultural residue, that can be utilized as a source of platform chemicals for biofuels. (Machado et al. 2016)

One of the most promising platform chemicals that can be produced from lignocellulosic biomass is furfural. Furfural is a valuable compound that can be utilized in several ways, so developing a method for its detection is the first step in the process of collecting it from biorefinery wastewaters.

HS-GC/MS is an analytical method for detecting volatile organic compounds. It combines gas chromatography and mass spectrometry, while using a headspace sampler. Separation of analytes in gas chromatography is based on their interaction with the stationary phase and mobile phase. In a mass spectrometer, ions are separated by their mass-to-charge ratio. Headspace sampling is a technique where a sample is vaporized inside a closed vial, removing a need for filtering and other sample preparation.

The objective of the bachelor's thesis was to develop an analytical method for determining furfural from wastewater samples with a new HS-GC/MS instrument in Materials Science and Environmental Engineering unit at Tampere University. An analytical method for wastewater samples containing furfural had been previously used with an old GC/MS instrument in the laboratory, and a comparable method for the new instrument was needed. The goal was to promote recovery of furfural from biorefinery wastewater as a part of a larger project. The purpose of the thesis was to find optimal parameters for the method by running assays and researching literature sources.

2 THEORETICAL BACKGROUND

2.1 Wastewater analysis

Selection of the analytical technique for wastewater analysis depends on the sample matrix and the target analyte. Domestic and industrial wastewaters contain different contaminants. While domestic wastewater contains traces of a wide variety of substances, industrial wastewater has higher concentrations of specific pollutans. (Vysokomornaya, Kurilenko & Shcherbinina 2015) Typical pollutants in industrial wastewater are heavy metals, phenols, and phenolic compounds. (Ahmed, Thakur, Goyal 2021)

Common instrumental techniques for organic analytes are gas chromatography and high-performance liquid chromatography. For compounds that are highly polar, prone to thermal decomposition, or have low volatility, HPLC technique should be selected. (Dean 2003, 185) A wide variety of organic compounds have been determined with gas chromatography. Some examples of compounds that can be detected from wastewaters with gas chromatography are polyaromatic hydrocarbons, organosulfuric compounds such as hydrogen sulfide, aldehydes, and phenols. (Crompton 2003, 363-369)

Combination of gas chromatography and mass spectrometry is used for wide variety of environmental analyses – common applications are drinking water and wastewater analyses. Both volatile and semi-volatile compounds in water can be detected with GC/MS technique. Pollutants such as furans and halogenated hydrocarbons are common target analytes in GC/MS wastewater analysis. (McMaster 2008, 96)

2.2 Furfural

Furfural ($C_5H_4O_2$) consists of a furan ring that has an aldehyde group attached to it. The structure of the compound appears in figure 1.

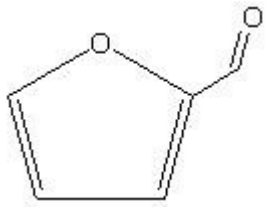


FIGURE 1. Structure of furfural, drawn with ChemSketch.

Furfural is a colorless or yellowish liquid that turns brown when exposed to air and light. It has a strong, almond-like odor. (PubChem 2022) Furfural causes skin and eye irritation, is toxic when swallowed and fatal when inhaled, and might be carcinogenic. Furfural is harmful to aquatic life. (Sigma Aldrich 2022) Primary properties of the compound are presented in table 1.

TABLE 1. Chemical properties of furfural (ILO & WHO 2021, Sigma Aldrich 2022, Pubchem 2022)

Molecular formula		$C_5H_4O_2$	ILO & WHO
CAS number		98-01-1	ILO & WHO
Molecular weight	g/mol	96,08	ILO & WHO
Boiling point	°C	162	ILO & WHO
Melting point	°C	-36,5	ILO & WHO
Flash point	°C	60	ILO & WHO
Relative density		1,16	ILO & WHO
Solubility in water	g/100 ml at 20 °C	8,3	ILO & WHO
Vapor pressure	kPa at 20 °C	0,23	Sigma Aldrich
Henry's law constant	atm-m^3/mol	$3{,}8 \cdot 10^{-6}$	Pubchem

According to Directive 2010/75/EU, a compound with a vapor pressure of at least 0,01 kPA at 20 °C is considered a volatile organic compound. (EUR-Lex n.d.) As shown in table 1, vapor pressure of furfural exceeds this limit, making it a volatile organic compound.

Furfural is derived from carbohydrates in lignocellulosic biomass like sugarcane bagasse and corn cobs, and it is a platform chemical for multiple high value compounds including furfuryl alcohol. (Yong et al. 2022) Furfuryl alcohol can be further converted into butyl levulinate that is a promising biofuel additive. (Peng,

Tao & Wu 2016) Other examples of fuel additives furfural can be converted into are 2-methylfuran, furan and tetrahydrofuran. (Ahmad et al. 2022)

Furfural analysis can be performed with techniques that detect organic compounds. HPLC can be used to determine furfural from different types of samples, such as food and beverage samples (Jeuring & Kuppers 1980; Kalal et al. 2007) and biomass hydrolysate. (Li et al. 2017) Similarly, GC/MS technique can be used for furfural detection in foods and beverages (Gaspar & Lopes 2009) and environmental samples, such as lignocellulosic hydrolysate. (Hu et al. 2015) When considering GC/MS analysis, a polar gas chromatography column should be chosen for analysis, because furfural is relatively polar due to its aldehyde group.

2.3 Introduction to HS-GC/MS technique

HS-GC/MS refers to a technique where a headspace sampler is used with a combination of a gas chromatograph and a mass spectrometer. A headspace sampler is an alternative to a conventional liquid sampler. After a sample has been heated in headspace oven and injected, analytes are separated in the GC column and ionized in the mass spectrometer. Ionized analytes are then separated by a mass analyzer and detected by an electron multiplier. Structure of a HS-GC/MS system is displayed in figure 2.

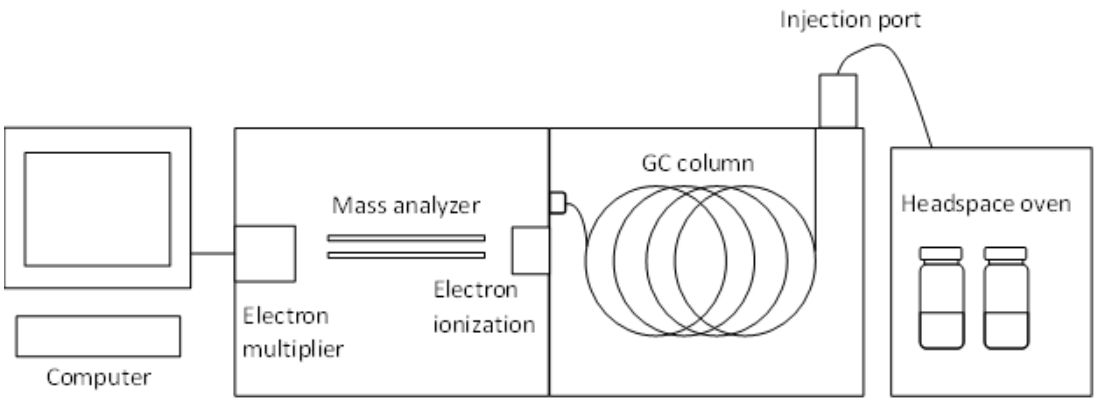


FIGURE 2. Structure of a HS-GC/MS system. (Hussain & Maqbool 2014, edited)

2.3.1 Headspace technique

Compared to a traditional GC sampling, a headspace sampler is a cost-effective and fast technique for analyzing volatile or semi-volatile organic compounds, as it allows the analysis of solid and liquid samples without extraction or other time-consuming sample preparation. Instead of injecting a liquid sample into the gas chromatogram column, a small amount of a liquid or solid sample is placed in a closed glass vial. While heated in the headspace sampler, volatile compounds rise above the sample material inside a vial and mix with the gas phase. When volatile compounds have diffused into the headspace, equilibrium is reached, and a small volume of aliquot is injected with a needle and transferred to the column. (Anderson, Berthod, Pino, Stalcup 2016, 814) Diffusion of volatile compounds into headspace is demonstrated in figure 3.

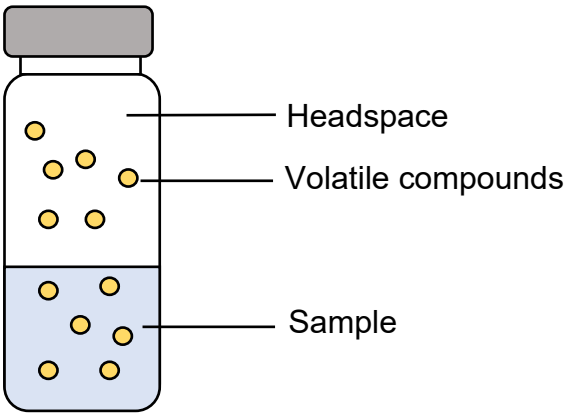


FIGURE 3. Equilibrium in a headspace vial. (Green 2005, edited)

Some central parameters in headspace sampling technique are equilibration time and temperature, and vial pressurizing pressure. Equilibrating temperature refers to the temperature the sample vial is heated in, and equilibration time is the duration of time the sample vial is heated for. Equilibration temperature should be high enough to obtain decent sensitivity and shorter equilibration time, but high temperatures can sometimes cause degradation of vial septa and therefore lead to contaminations. When using a high equilibration temperature, the internal vial pressure will also increase, which should be taken into consideration to avoid possible breakage of septa or vials. Equilibration time depends on the properties

of sample components - highly volatile components start to migrate to the headspace sooner, sometimes already during the sample preparation. Pressurizing pressure of the vial must be higher than the internal pressure of the vial to prevent backflow of the headspace gases, but it should not exceed the maximum pressure that the vial can withstand. (Henshaw 2012)

One matter to consider when developing a method for HS-GC/MS is choosing the correct sample volume. Using larger sample volumes does not necessarily increase sensitivity, because the more there is sample material in the vial, the smaller the headspace volume becomes. (Kott 2010) Additionally, sample line and transfer line temperatures must be adjusted correctly. Generally, when an analyte is travelling from the headspace to the GC, temperature should be increasing at each point to minimize condensation. (Goodman 2008) For example, transfer line temperature should be slightly higher than sample line temperature to avoid issues such as sample carryover. (Wichitnithad et al. 2021) Sample carryover refers to a phenomenon where an analyte from a previous sample appears in subsequent samples during a run. One situation that increases the risk of carryover is when blanks or samples with low concentrations are placed after high-concentration samples in a sequence. (Hughes et al. 2007)

Salting-out technique can be used to increase the concentration of target analytes in the headspace by increasing the transfer of volatile compounds from liquid phase from gas phase. This can be done by adding inorganic salts to the sample matrix. (Perkin Elmer 2013-2014)

2.3.2 Gas chromatography

Gas chromatography is a tool for analysis of organic compounds. To be suitable for gas chromatography, analytes must withstand high temperature without breaking down. (Sparkman, Penton & Kitson 2011, 15) When using a conventional injector in gas chromatography, liquid samples are injected to the column. Choosing an injection mode depends on the sample – split injection refers to a technique where only a small quantity of the sample is injected, and it should be used for concentrations higher than 100 µg/ml. Splitless injection mode

increases sensitivity, because it utilizes the whole sample, so it is recommended especially for trace analysis. A disadvantage of splitless injection mode is that the long time spent in the injector might lead thermal breakdown of some compounds. (Sparkman, Penton & Kitson 2011, 19-21)

When using a conventional liquid injector, the role of a liner is to vaporize a liquid sample before it travels into the column. Therefore, the liner should be selected by considering what is the suitable internal diameter, coating, and shape for the analysis in question. The shape of the liner depends on the injection mode – a linear liner is suitable for split mode, and a tapered liner for splitless mode. There are packed and unpacked liners available, and different packing materials are suited for different types of analytes. (Anderson et al. 2016, 809-810)

One of the main considerations in gas chromatography is selection of the column. In gas chromatography, a carrier gas is used as the mobile phase that transfers the sample through the column, which is a tube coated by the stationary phase, usually siloxanes. (Poole 2012, 80) The carrier gases most commonly used for gas chromatography are helium and hydrogen. (Sparkman, Penton & Kitson 2011, 15) Nitrogen is often not a good option because of its high viscosity, which limits the efficiency of the gas at high flow rate. (McMaster 2008, 35)

The separation of components is based on their retention time, which refers to the time a compound has taken to pass through the column. Several factors such as flow rate, length of the column and temperature conditions affect the retention time. (Sparkman, Penton & Kitson 2011, 50)

After elution the component travels into a detector. There are several types of detectors available for different sample types. A mass spectrometer is commonly used as a detector. Another popular choice is a flame ionization detector (FID), and it is suitable for the most organic compounds. It detects ions that are formed when the sample material is being combusted in a hydrogen flame, and a flow of air is used to promote the combustion. To prevent condensation, the temperature of the detector must be at least 125 °C. Ions produced in the combustion form a current, which produces the signal. (McNair, Miller, Settle 2009, 115)

A differential thermal conductivity detector (TCD) is one of the oldest detector types, and it responds to substances that have a different thermal conductivity than the carrier gas. (Bansal 2005, 211) An electron capture detector (ECD) is sensitive to compounds with a high affinity for electrons. It is mostly used for detection of environmental pollutants like chlorinated pesticides. A weakness of this detector is its low linear range. (Bansal 2005, 214-215)

2.3.3 Mass spectrometry

A gas chromatograph is often paired with a mass spectrometer, which in this case functions as a detector. A downside of using a gas chromatograph alone is that GC detectors mostly rely on retention time only when identifying sample compounds. Combination of a gas chromatograph and a mass spectrometer can provide more information about the sample than a gas chromatograph alone, therefore being more reliable for both qualitative and quantitative analysis. (McMaster 2008, 4-5)

Separation in the mass spectrometer is based on mass-to-charge (m/z) ratio of charged particles, and therefore the sample needs to be ionized. The most common ionization method is electron ionization (EI), followed by chemical ionization (CI) and field ionization (FI). In electron ionization, sample is introduced to the ion source. Filament emits free electrons, and using typically charge of 70 eV, electrons are accelerated towards the trap, where they interact with sample molecules. Molecules lose an electron, therefore becoming cations. (Lebedev 2011, 5-6, 21)

After ionization, mass analyzer separates the ions based on their unique m/z ratio. Quadrupoles, ion traps and time of flight mass analyzers are often used as a mass analyzer with GC/MS technique. The quadrupole analyzer is comprised of four metal rods. Ions travel between the rods and high frequency voltage is applied to the rods to filter the ions so that only ions with a particular m/z ratio will reach the detector of the MS. (McNair, Miller, Settle 2009, 239-240)

Mass spectrometers typically use an electron multiplier to detect ions. When the ions hit the surface of the dynode, electrons are released, and the signal gets enhanced by every further collision. The strength of the signal is therefore proportionate to the number of ions arriving to the detector. (Lebedev 2011, 18)

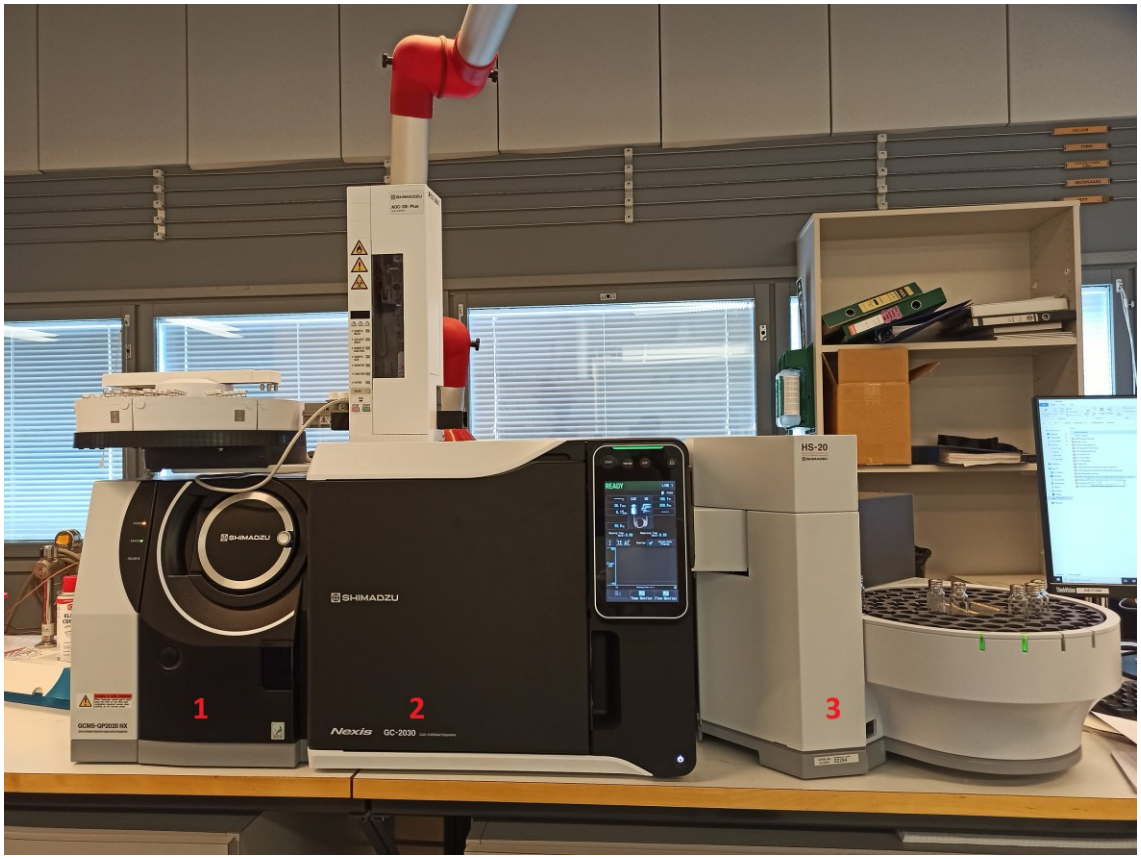
A mass spectrometer can be operated in either SIM or SCAN mode. In the scanning mode (SCAN), which is often used for qualitative analysis, a mass spectrometer detects all the ions in the selected mass range. Single-ion monitoring mode (SIM) is used when only selected target ions need to be detected. In this case, the sensitivity of the detection is also increased. (McMaster 2008, 12-14)

3 METHOD DEVELOPMENT

3.1 Instrumentation and reagents

Analytical grade furfural (99% purity) was purchased from Sigma-Aldrich.

The method was developed for a gas chromatograph (Shimadzu Nexis GC-2030) combined with a mass spectrometer (Shimadzu GCMS-QP2020) and a headspace sampler (Shimadzu HS-20). The system is presented in the picture 1.



PICTURE 1. Shimadzu HS-GC/MS system the method was developed for. The system consists of three components: GCMS-QP2020 (1), Nexis GC-2030 (2) and HS-20 (3). (Heini Laine 2022)

Assays were carried out with a ZB-WAXplus™ column, manufactured by Phenomenex. The length of the column is 30 m, it has an inner diameter of 0,25 mm and film thickness of 0,25 µm. The temperature limits of the column are minimum of 20 °C and maximum of 250/260 °C. ZB-WAXplus™ is suitable for

aqueous samples and polar compounds with a relatively low boiling point, so it is an appropriate choice for wastewater samples containing furfural. (Phenomenex Inc. 2022) Screw cap headspace glass vials of 20 ml from Shimadzu were used for the assays.

Parameters from literature were applied for the assays and modified during the method development process, as discussed in the later chapters. Peaks were integrated automatically with GCMS solution Version 4.53 by Shimadzu Corporation, except in cases the peak was not detected by the software and therefore it had to be done manually. Peak area was chosen for quantification. SCAN mode was chosen for all the assays to ensure the identification and separation of the target analyte in the beginning of the method development. NIST database was used for the compound identification from the mass spectrum. Only matches higher than 70% similarity index were included.

3.2 Method development process

Furfural standards with different concentrations ranging from 50 µmol/l to 60 000 µmol/l were prepared and analyzed during the method development process. Milli-Q water was used for blanks. Parameters of the methods discussed in this chapter are presented in appendix 1, table 5. Equations applied for preparation of solutions and standards are presented in appendix 2.

Method 1 was created with an aim to test if a furfural peak could be detected from a standard containing a known amount of furfural. Parameters from literature (Hu et al. 2015) and an old method used for the previous instrument in the laboratory were applied. As instructed in Shimadzu HS-20 manual, equilibrating temperature should be at least 20 °C lower than boiling point of the solvent. Appropriate equilibrating temperature range for water samples is 60-80 °C. (Shimadzu 2012-2013, 135) Therefore, HS equilibrating temperature was set to 80 °C to ensure peak detection. Equilibration time was set to 2 minutes.

To test Method 1, 120 000 µmol/l furfural solution was prepared, and the first standards analyzed were 12 000 µmol/l and 60 000 µmol/l, followed by a blank.

Standard concentrations were chosen based on the old standard curve that had been used for the old HS-GC/MS system in the same laboratory.

After testing Method 1, several parameters were changed for the Method 2, because the results showed poor separation of furfural peaks and sample carryover. Knowing the molecular mass of furfural is 96,08 g/mol, the mass spectrometer scan range was set to 40-200 m/z to decrease the appearance of overlapping peaks. Considering that retention of furfural occurred when the temperature was around 110-150 °C, the gas chromatogram oven temperature program was adjusted so that the temperature rise was slower around the furfural retention.

Method 3 was developed to further improve peak separation. M/z range was set to 50-200 to prevent peak overlap. GC oven temperature program was optimized further to avoid unnecessarily long analysis time. After sufficient peak separation was achieved with Method 3, effects of different headspace sampler conditions were tested in progress analysis mode. The objective was to examine the change in peak area when testing different values of a single parameter. Equilibrating temperatures in the range of 60-80 °C, and equilibrating times in the range of 1-16 minutes were tested in a progress analysis mode. Additionally, the effect of sample volume to peak area was tested by pipetting different volumes of furfural standard into headspace vials. Volumes in the range of 100-2000 µl were tested in the experiment.

3.3 Minimizing sample carryover

After carryover issue was noticed, The GC column was baked out for 120 minutes in 230 °C and different temperature programs were tested to find out if the GC column was the source of carryover. Total flow rate and purge flow rate were increased. Any of these attempts did not produce significantly better results.

Further variations of Method 3 were created to be used for troubleshooting tests when trying to find the cause of persistent carryover. Temperatures of HS sample line and transfer line were set to 200 °C to minimize condensation. Longer needle

flush times, 8 and 16 minutes, were also tested. In addition to this, a variation of Method 3 with 8 minutes of needle flush time was applied on a test where 10 empty vials were placed after a furfural standard.

As the headspace sampler was already identified as the source of carryover, headspace parameters of methods 1-3 were compared in order to find out which headspace conditions had produced the least carryover. Based on these observations, Method 4 was developed to fix the carryover issue that had still been present when using Method 3. In addition to lowering equilibrating temperature and increasing needle flush time, injection time was also decreased from 1,0 minutes to 0,5 minutes.

3.4 Wastewater sample analysis

After Method 4 was tested and proved to be working, calibration standards were prepared. During the method development, approximate furfural concentration of the wastewater samples was estimated by running a preliminary test with wastewater samples and comparing their peak area to the peak area of standards of 12 000 µmol/l and 60 000 µmol/l. Based on this, furfural concentration of the wastewater samples was estimated to be around 50 µmol/l.

Seven standards in the range of 5-100 µmol/l were prepared. A working solution of 25 mmol/l was prepared by pipetting 207 µl of 99% furfural into a volumetric flask of 100 ml. After this, a stock solution of 100 µmol/l was prepared from the working solution by pipetting 400 µl of the working solution into a volumetric flask of 100 ml.

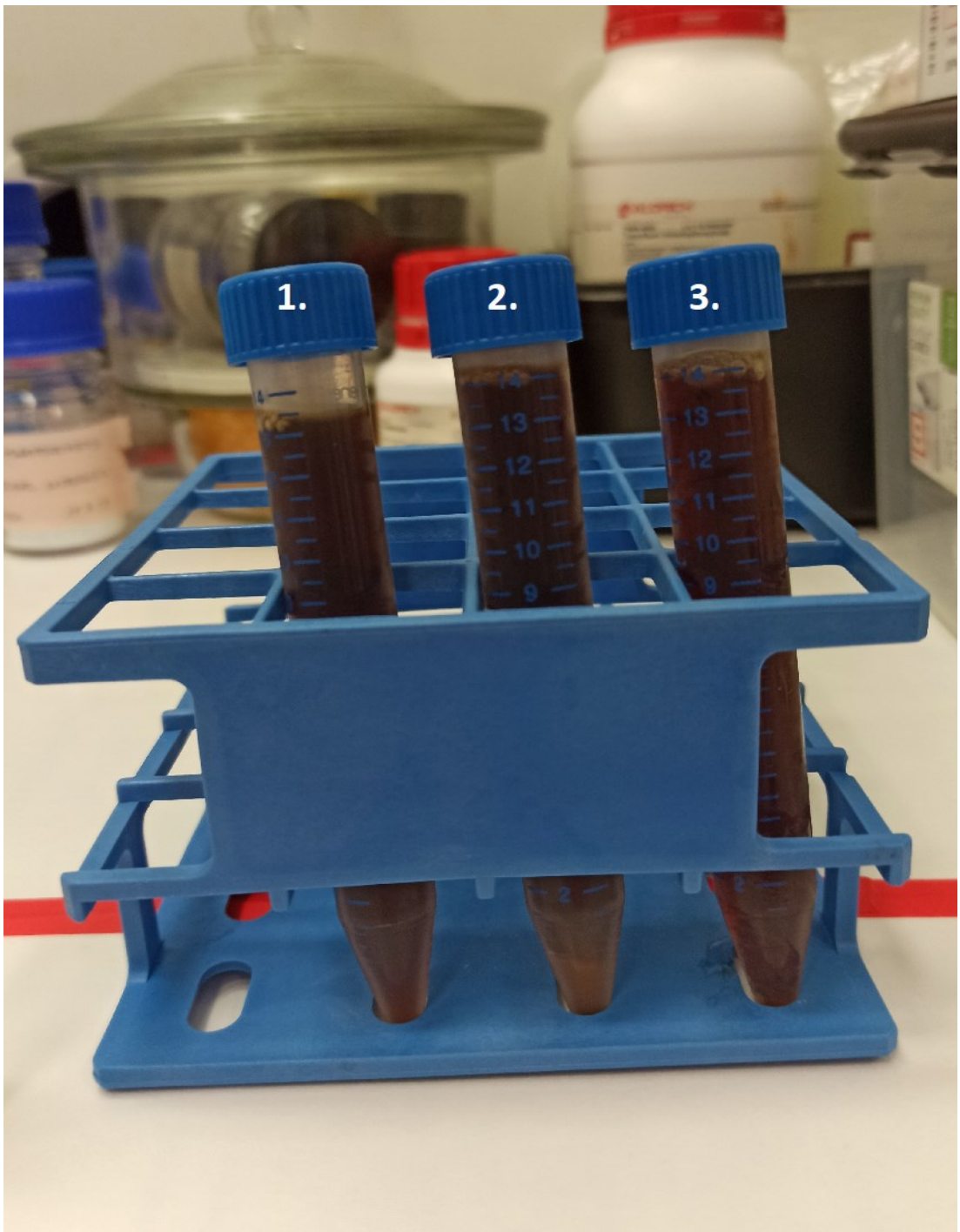
When preparing calibration standards, the objective was to obtain coefficient of determination of at least 0,9. Seven calibration standards were prepared as triplicates by pipetting stock solution and milli-Q water into headspace vials with the final volume of 500 µl, as presented in table 2.

TABLE 2. Preparation of the calibration standards

M	V (stock)	V (milli-Q)
µmol/l	µl	µl
5	25	475
10	50	450
15	75	425
25	125	375
50	250	250
75	375	125
100	500	0

Wastewater samples were obtained from a biorefinery, but exact origin of the samples could not be disclosed due to confidentiality. Each sample was taken from a different canister, and they were known to possibly contain traces of furfural.

Sample analysis was performed with Method 4. Samples 1-3 were prepared by pipetting 500 µl of undiluted sample material to headspace vials. Sample volume was chosen based on tests conducted during the method development. Three wastewater samples are presented in picture 2 and a sample pipetted in a headspace vial in picture 3. The dark color of the samples does demonstrate how filtering would have been necessary if method had been developed for HPLC instead of HS-GC/MS.



PICTURE 2. Wastewater samples 1-3. (Heini Laine 2022)

PICTURE 3. 500 µl of the sample in a headspace vial. (Heini Laine 2022)

4 RESULTS

4.1 Results from the method development phase

Results from the run using Method 1 showed that the method was not adequate, because the peak was not clearly separated due to several overlapping peaks with retention times at 7-8 minutes, as shown in figure 4. Furfural could not be identified reliably for this reason. However, in a milli-Q blank that was ran after the samples, furfural carryover peak was detected with a retention time of 7,646 minutes.

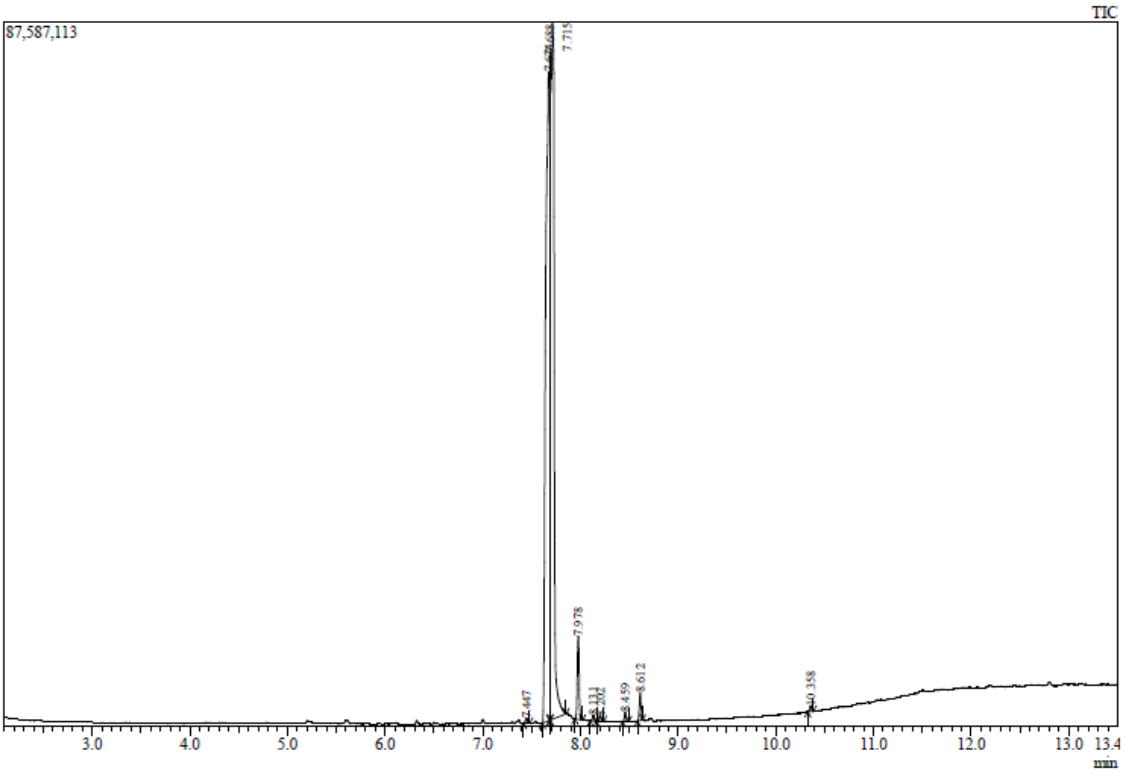


FIGURE 4. Retention time of furfural is approximately at 7,6 minutes.

The chromatogram shows that the baseline is rising during the temperature program due to relatively high final temperature.

Method 2 provided slightly better separation of furfural peaks. Retention time of furfural was 10,777 minutes, but acetic acid appeared at 10,340 minutes. Successful separation of furfural peaks with no peak overlap was finally achieved with Method 3.

Different headspace sampler conditions were tested as a part of the method development process. Peak area was the largest with sample volume of 500 µl, but the difference between different sample volumes was not significant. Effects of different values for equilibrating time and equilibrating temperature on peak area are demonstrated in figures 5 and 6.

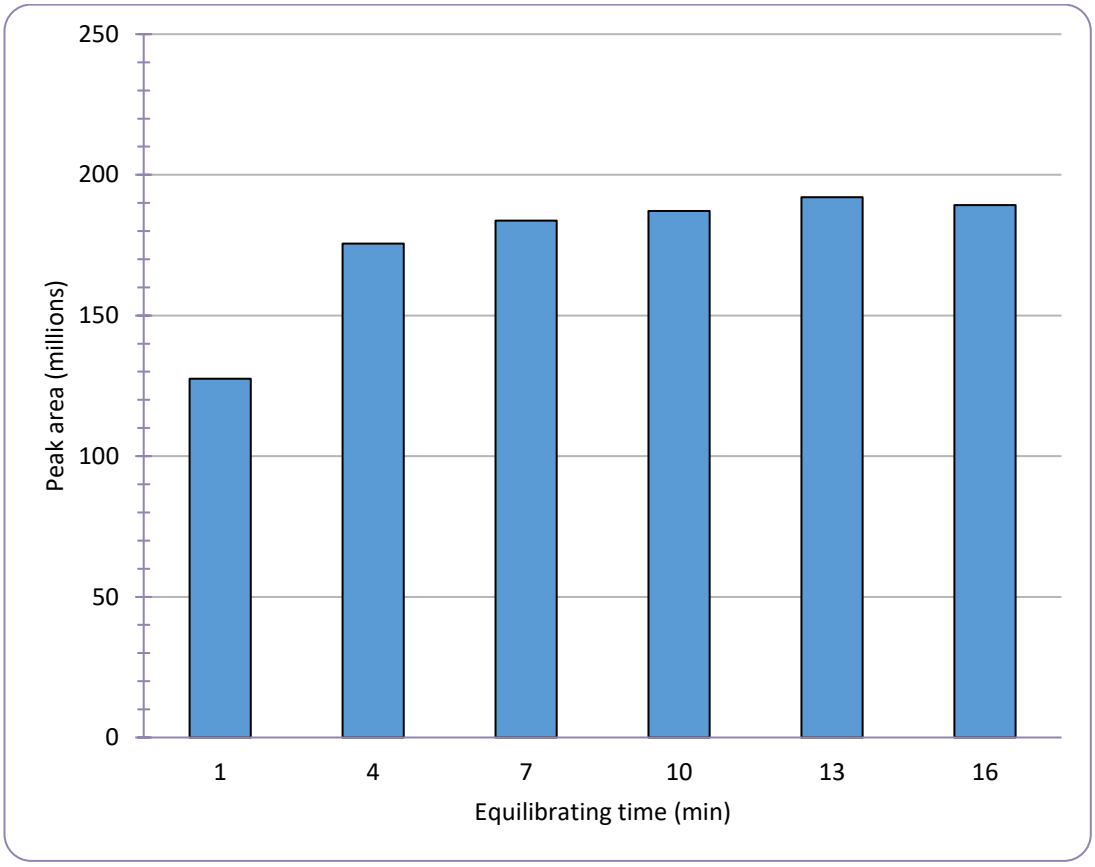


FIGURE 5. Effect of equilibrating time on peak area.

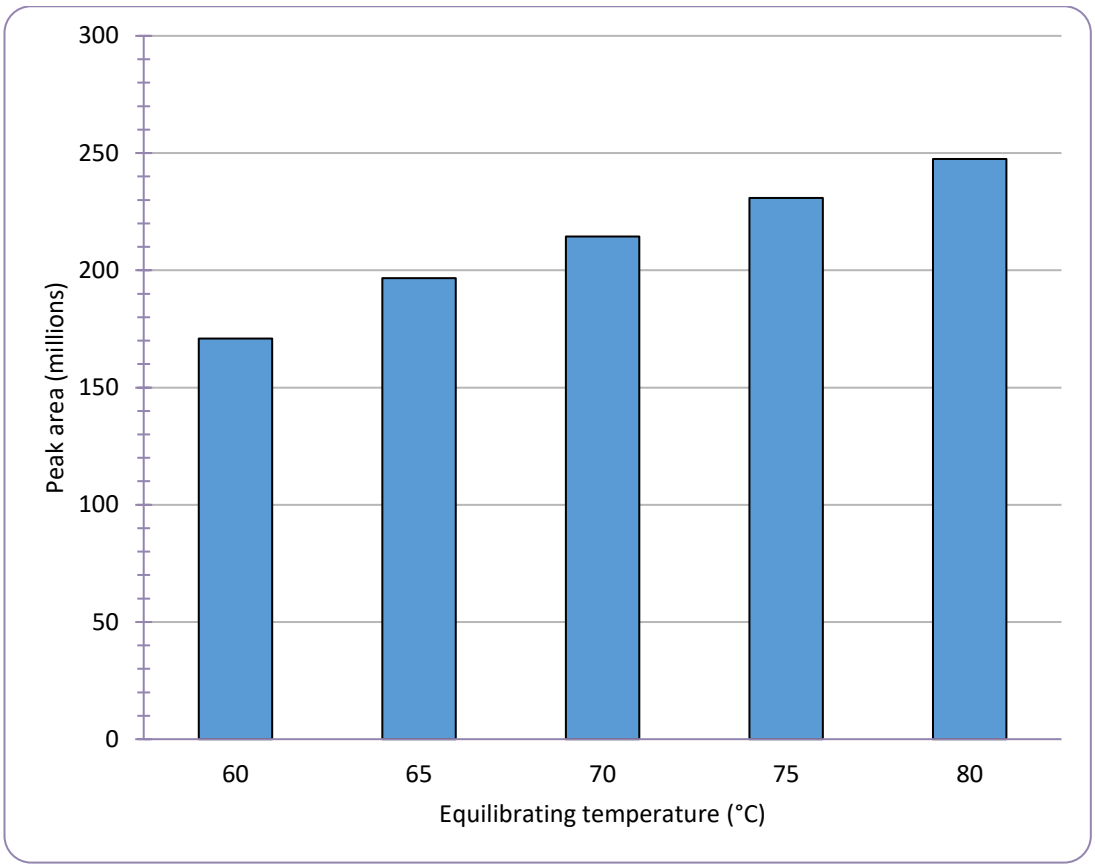


FIGURE 6. Effect of equilibrating temperature on peak area

Equilibration time of 13 minutes resulted in largest peak area, but when comparing the peak area achieved with equilibration time of 4 minutes, the difference is not significant. Increasing equilibration temperature has an incremental effect on the peak area, and largest peak area was achieved with 80 °C.

Method 4, developed based on the findings from all the previous tests and carryover tests, was finally tested. Carryover was not detected anymore, furfural peak was clearly separated, and similarity index for furfural was 98%. Chromatogram for a furfural standard analyzed with Method 4 is shown in figure 7.

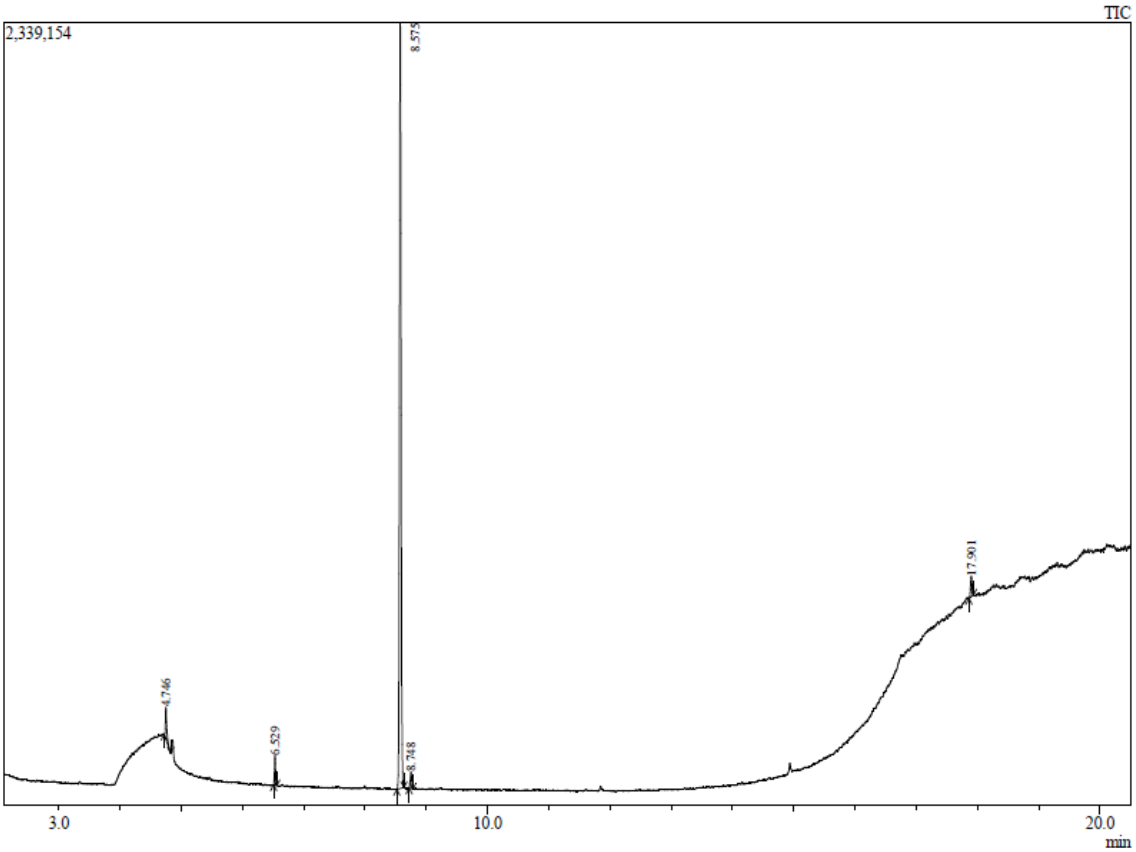


FIGURE 7. Chromatogram of a furfural standard of 100 µmol/l analyzed with Method 4. Retention time of furfural is 8,6 minutes.

4.2 Sample carryover tests

Sample carryover tests were run with variations of Method 3, using milli-Q water as blanks. Carryover percentage ranged from 1,97% to 2,57%, being lowest when needle flush time was 16 minutes and sample line and transfer line temperatures were 150 and 160 °C, respectively.

When running ten empty vials as blanks after a furfural standard, carryover percentage was 0,15% at its highest. Peak area decreased over a series of blanks so that furfural peak was not detectable after four blanks. Decrease of carryover is demonstrated in figure 8.

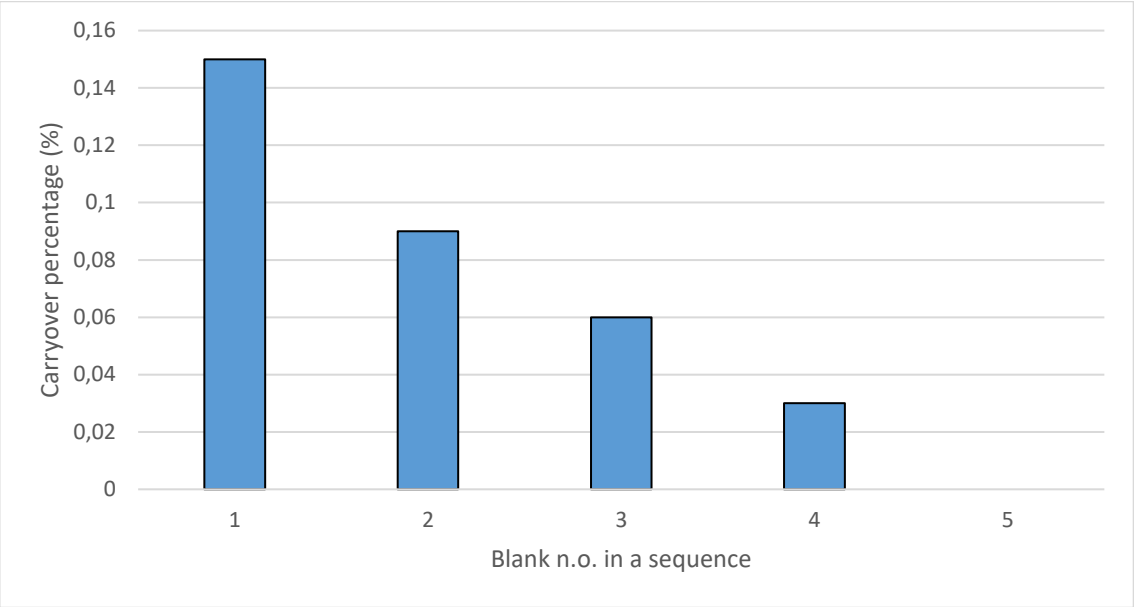


FIGURE 8. Decrease of carryover percentage over a series of blanks.

Compared to tests with milli-Q blanks, it was noted that using empty vials as blanks reduced carryover for an unknown reason.

When comparing different methods that had been tested during method development, it was noted that carryover had been lowest with equilibrating temperature of 60 °C. Additionally, increasing needle flush time to 16 minutes had decreased carryover slightly.

4.3 Results of wastewater sample analysis

Seven standards and three undiluted wastewater samples from a biorefinery were analyzed with Method 4. After the standards were ran as triplicates, the mean value of peak area was calculated for each standard. Results are presented in table 3.

TABLE 3. Calculated mean for peak area of each calibration standard.

Concentration (µmol/l)	Peak area
5	210691,6667
10	410434,3333
15	622452,3333
25	1039630,667
50	2132062,667
75	3228642,667
100	4406752,333

The calibration curve presented in figure 9 was created with Microsoft Excel based on the values shown in table 3. As the goal value for coefficient of determination was 0,9 at a minimum, results can be considered as reliable.

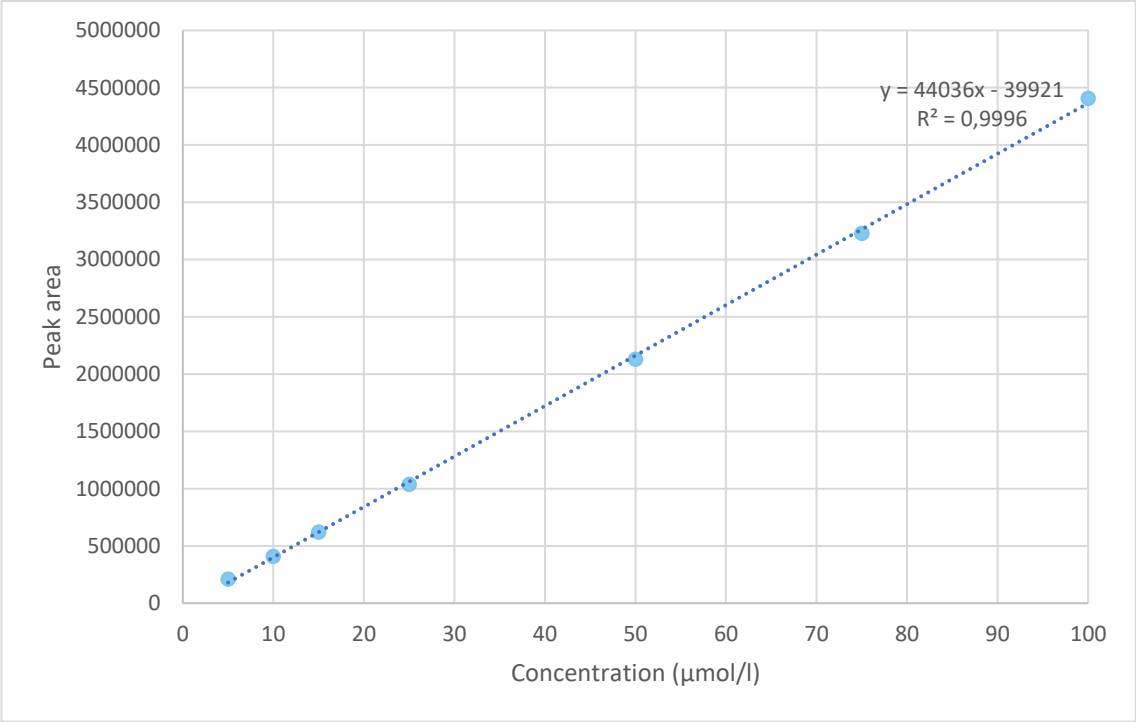


FIGURE 9. Furfural calibration curve.

Furfural was identified in all three samples. Furfural concentration of the samples was calculated from the slope of the calibration curve displayed in figure 9 and are presented in table 4.

TABLE 4. Calculated furfural concentration for samples 1-3

Sample	Peak area	Concentration (µmol/l)
1	93581	<5
2	565151	13,74
3	132364	<5

Concentrations of samples 1 and 3 were lower than expected, falling below 5 µmol/l. Chromatograms of the samples are presented in figures 10-12, where retention time of furfural is 8,6 minutes. Because samples were analyzed using SCAN mode, several other compounds were detected as well. Some compounds detected in the samples 1-3 are listed in appendices 3 and 4, in which they are presented in tables 6-8.

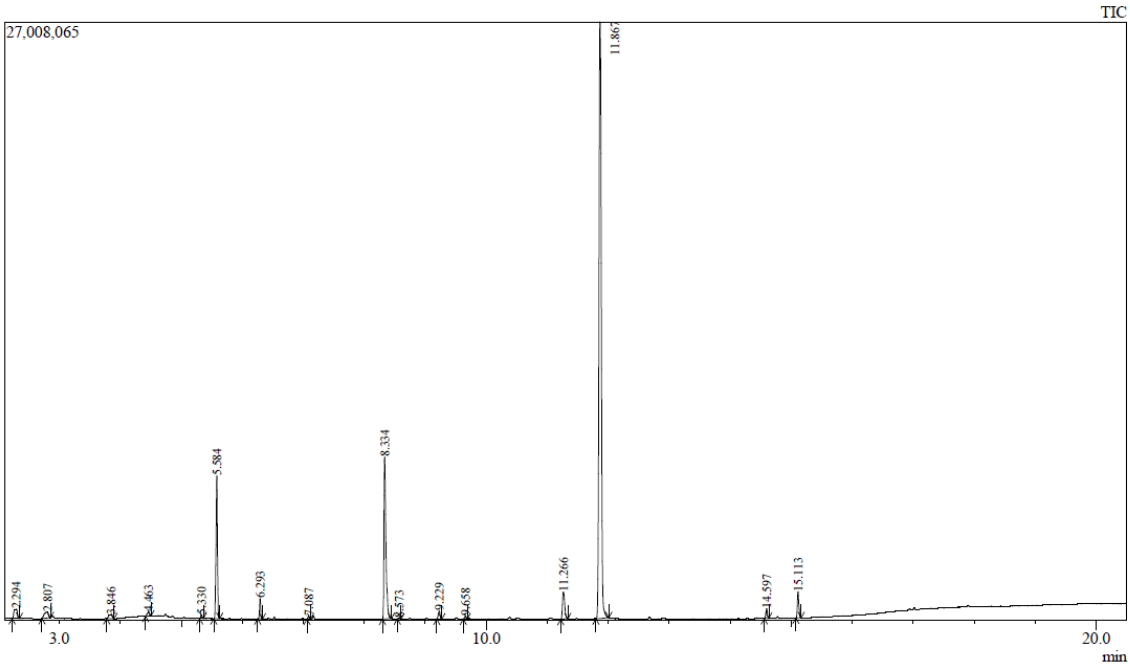


FIGURE 10. Sample 1 chromatogram.

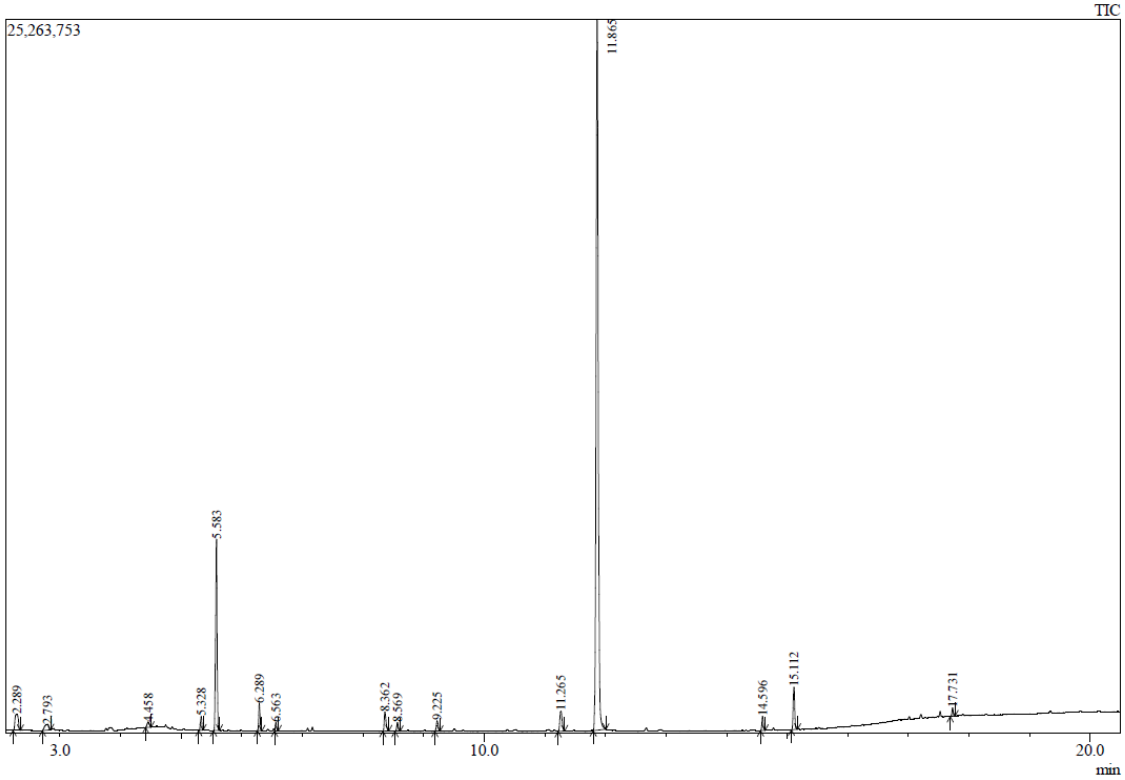


FIGURE 11. Sample 2 chromatogram.

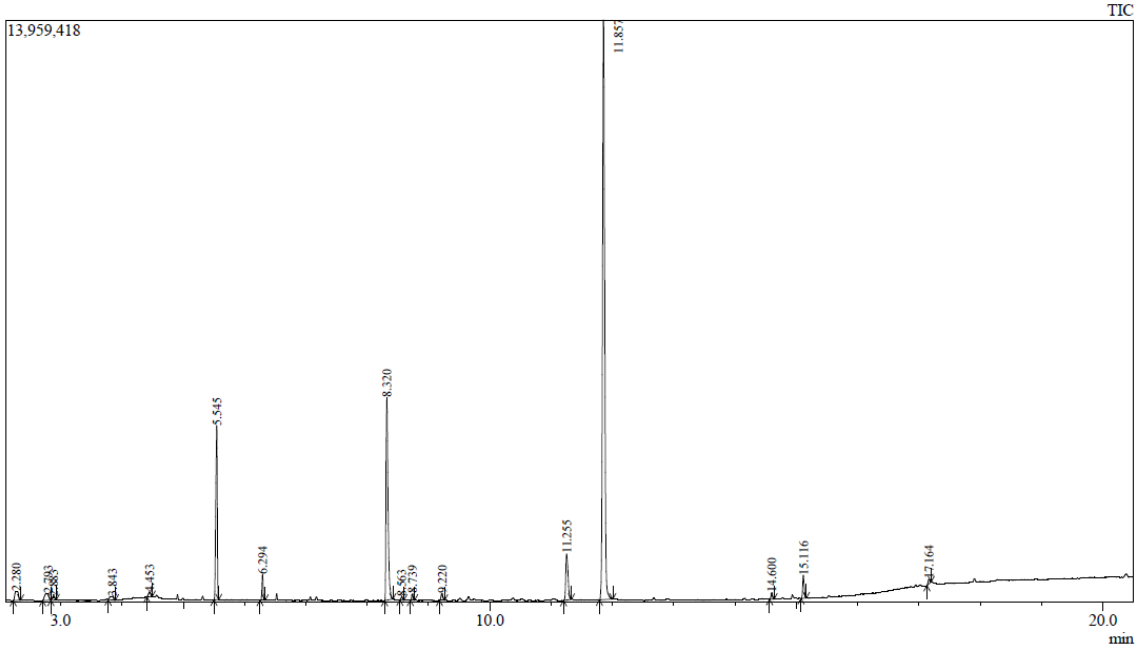


FIGURE 12. Sample 3 chromatogram.

5 DISCUSSION

The objective of the thesis was to develop a method for furfural analysis from wastewater samples with HS-GC/MS technique. The purpose of the thesis was to find optimal instrument parameters for the method by testing different test setups. Even though a functional method was developed, instrument difficulties and time constraints prevented full completion of the initial goal, as concentration of all three wastewater samples was not determined. Due to aforesaid issues, the method was not validated.

The first major problem was recurring appearance of furfural peaks in blanks posed a challenge to the method development process. Due to low furfural concentration in the samples, minimizing carryover was considered essential to provide reliable results. Carryover issues in gas chromatography are often caused by an injector or a contaminated liner. (Sparkman, Penton & Kitson 2011, 499) However, when using a headspace sampler, the situation is more complex to solve, as the troubleshooting sections in the literature are mostly describing issues caused by conventional liquid injectors. The GC column was baked out and several different GC temperature program changes were attempted, but carryover persisted. The cause of the carryover was traced to the headspace sampler, as changing headspace parameters had a noticeable effect on the furfural peak area in blanks.

As one potential cause for carryover peaks is condensation caused by low temperatures in the headspace system, temperatures of sample line and transfer line were increased to 150 and 160 °C, which slightly decreased the carryover. However, temperature of 200 °C for both sample line and transfer line did not produce additional decrease in carryover. Managing to finally create Method 4 required a combination of changes – keeping sample line and transfer line temperatures at 150 and 160 °C, lowering equilibrating temperature to 70 °C, increasing needle flush time to 16 minutes, and decreasing injection time to 0,5 minutes.

While Method 4 was otherwise satisfactory, the range covered by calibration standards was not adequate for the wastewater samples. In a previous method for furfural analysis at Tampere university, the linear range had been 5-25 mmol/l, but after running preliminary tests for the wastewater samples, furfural concentrations were estimated to be around 50 µmol/l. However, after preparing calibration standards in the range of 5-100 µmol/l and running the samples, concentrations were significantly lower than estimated, and therefore concentration of samples 1 and 3 could not be determined. If more time had been available for the project, different range of calibration standard concentrations could have been tested. As very low concentrations were detected from the samples, determining limit of detection for the method could be useful. In addition to this, salting out technique could have been attempted with the calibration standards and wastewater samples. (Perkin Elmer 2013-2014) In the beginning of the project salting out was not considered as necessary, because sample concentrations were estimated to be high enough to produce a strong enough signal.

Sample volume could have possibly been higher to make sample preparation easier. Sample volumes ranging from 100 - 2000 µl were tested, but not with the wastewater samples. To develop a finalized method, testing different sample volumes and equilibrating times with the actual samples could be possibly useful. Considering SIM mode instead of SCAN mode to increase sensitivity might also be worth considering as a next step, as SIM mode is more suitable for quantitative analysis, especially when concentrations are low. (McMaster 2008, 14)

An interesting observation from the samples was that furfuryl ethyl ether was detected, as shown in appendix 3. Furfuryl ethyl ether is one of the compounds that have been researched as potential fuel additives. (Ahmad et al. 2022) The compound has been considered even as a potential replacer of petroleum diesel. (Mulik, Niphadkar & Bokade 2020) Therefore, possible future considerations include developing the method further to determine other compounds in addition to furfural.

REFERENCES

Ahmad, F., Kalam, M., Zhang, Z., Masjuki, H. 2022. Sustainable production of furan-based oxygenated fuel additives from pentose-rich biomass residues. Read 28.11.2022. https://www.sciencedirect.com/science/article/pii/S2590174522000459

Ahmed, J., Thakur, A., Goyal, A. 2021. Industrial Wastewater and Its Toxic Effects. Chapter 1 in Biological Treatment of Industrial Wastewater, 2022. Read 25.11.2022. https://pubs.rsc.org/en/content/chapterhtml/2021/bk9781839162794-00001?isbn=978-1-83916-279-4&sercode=bk

Anderson, J., Berthod, A., Pino, V., Stalcup, A. 2016. Analytical Separation Science, 5 Volume Set. John Wiley & Sons.

Bansal, K. 2005. Chromatography. Campus Books International.

Crompton, T. 2003. Chromatography of Natural, Treated and Waste Waters. Spon Press.

Dean, J. 2003. Methods for environmental trace analysis. John Wiley & Sons.

EUR-Lex. Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control). Read 28.11.2022. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010L0075-20110106

Gaspar, E., Lopes, J., 2009. Simple gas chromatographic method for furfural analysis. https://pubmed.ncbi.nlm.nih.gov/18976770

Goodman, W. 2008. VOC Analysis of Water-Based Coatings by Headspace-Gas Chromatography. PerkinElmer Inc. https://www.s4science.at/wordpress/wp-content/uploads/2018/10/VOC-Analysis-Of-Waterbased-Coatings.pdf

Green, J. 2005. HEADSPACE ANALYSIS | Static. Read 29.11.2022. https://www.sciencedirect.com/science/article/pii/B0123693977002545

Henshaw, J. 2012. Headspace Sampling: Instrumentation. LCGC Asia Pacific-03-01-2012, Volume 15, Issue 1, pages 27–32. https://www.chromatographyonline.com/view/headspace-sampling-instrumentation

Hu, H., Yang, X., He, T., Huang, L., & Cheng, L. 2015. Rapid Method for Simultaneous Determination of the Acetic Acid and Furfural Contents in Lignocellulosic Hydrolysate by Full Evaporation Headspace Gas Chromatography. https://pubs.acs.org/doi/abs/10.1021/acs.energyfuels.5b02072

Hughes, N., Wong, E., Fan, J., Bajaj, N. 2007. Determination of Carryover and Contamination for Mass Spectrometry – Based Chromatographic Assays. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2751487/

Hussain, S., Maqbool, K. 2014. GC-MS: Principle, Technique and its application in Food Science. Read 29.11.2022. https://www.semanticscholar.org/paper/GC-MS%3A-Principle%2C-Technique-and-its-application-in-Hussain-Maqbool/77cae8ddea08d6eb6c8d26e50bd19c63da7deda3

ILO & WHO 2021. Furfural. https://www.ilo.org/dyn/icsc/showcard.display?p_version=2&p_card_id=0276

Jeuring, H., Kuppers, F., 1980. High performance liquid chromatography of furfural and hydroxymethylfurfural in spirits and honey. Read 8.6.2022. https://pubmed.ncbi.nlm.nih.gov/7451383/

Kalal, H., Mahani, M., Maregheh, M., Chaloosi, M., 2007. HPLC Determination of Furfural after Preliminary Extraction to Aqueous Phase. Read 29.9.2022. https://www.tandfonline.com/doi/abs/10.1080/10826070701435087

Kott, L. 2010. Experimental Considerations in Headspace Gas Chromatography. Pharmaceutical Technology-05-02-2010, Volume 34, Issue 5. Read 25.8.2022. https://www.pharmtech.com/view/experimental-considerations-headspace-gas-chromatography

Lebedev, A. 2011. Comprehensive Environmental Mass Spectrometry. ILM Publications.

Li, J., Xu, Y., Zhang, M., Wang, D., 2017. Determination of Furfural and 5-Hydroxymethylfurfural in Biomass Hydrolysate by High-Performance Liquid Chromatography. Read 1.7.2022. https://pubs.acs.org/doi/pdf/10.1021/acs.energyfuels.7b02827

Machado, G., Leon, S., Santos, F., Lourega, R., Dullius, J., Mollmann, M., Eichler, P., 2016. Read 1.7.2022. Literature Review on Furfural Production from Lignocellulosic Biomass. https://www.scirp.org/journal/paperinformation.aspx?paperid=64775

McMaster, M. 2008. GC/MS: A practical User's Guide. Second edition. John Wiley & Sons, Inc.

McNair, H., Miller, J., Settle, F. 2009. Basic gas chromatography. John Wiley & Sons.

Mulik, N., Niphadkar, P., Bokade, V. 2020. Synthesis of ethyl furfuryl ether (potential biofuel) by etherification of furfuryl alcohol with ethanol over heterogenized reusable H1Cs2PW12O40 catalyst. Read 28.11.2022. https://www.researchgate.net/publication/339007305_Synthesis_of_ethyl_furfuryl_ether_potential_biofuel_by_etherification_of_furfuryl_alcohol_with_ethanol_over_heterogenized_reusable_H1Cs2PW12O40_catalyst

Perkin Elmer Inc. 2013-2014. An introduction to headspace sampling in gas chromatography: fundamentals and theory. Compiled by Tipler, A. Read 25.10.2022. https://resources.perkinelmer.com/corporate/pdfs/downloads/gde_intro_to_headspace.pdf.

PubChem. Furfural. Read 10.6.2022. https://pubchem.ncbi.nlm.nih.gov/compound/Furfural

Shimadzu 2012-2013. Head Space Sampler HS-20. Instruction manual.

Sigma-Aldrich. Furfural. A safety data sheet. 2022. Read 10.11.2022. https://www.sigmaaldrich.com/FI/pt/sds/aldrich/w248908

Sparkman, D., Penton, Z., Kitson, F. 2011. Gas Chromatography and Mass Spectrometry: a Practical Guide. Elsevier Science & Technology.

Peng, L., Tao, R., Wu, Y. 2015. Catalytic Upgrading of Biomass-Derived Furfuryl Alcohol to Butyl Levulinate Biofuel over Common Metal Salts. Read 28.11.2022. https://www.mdpi.com/2073-4344/6/9/143

Phenomenex Inc. 2022. ZB-WAXplus Phase Detail. https://www.phenomenex.com/Products/GCDetail/Zebron/ZB-WAXplus

Poole, C. 2012. Gas Chromatography. Elsevier.

Vitha, M. 2016. Chromatography: Principles and Instrumentation. John Wiley & Sons.

Vysokomornaya, O., Kurilenko, E., Shcherbinina, A. 2015. Major Contaminants in Industrial and Domestic Wastewater. Read 15.11.2022. https://www.researchgate.net/publication/282464855_Major_Contaminants_in_Industrial_and_Domestic_Wastewater

Wichitnithad, W., Sudtanon, O., Srisunak, P., Cheewatanakornkool, K., Nantaphol, S., Rojsitthisak, P. 2021. Development of a Sensitive Headspace Gas Chromatography–Mass Spectrometry Method for the Simultaneous Determination of Nitrosamines in Losartan Active Pharmaceutical Ingredients. Read 30.10.2022. https://pubs.acs.org/doi/full/10.1021/acsomega.1c00982

Yong, K., Wu, T., Lee, C., Lee, Z., Liu, Q., Jahim, J., Zhou, Q., Zhang, L. 2002. Furfural production from biomass residues: Current technologies, challenges and future prospects. https://www.sciencedirect.com/science/article/abs/pii/S0961953422001192

APPENDICES

Appendix 1. Parameters for tested methods

TABLE 5. Method parameters.

		Method 1	Method 2	Method 3	Method 4
HS-20	Equilibrating temp. (°C)	80	60	80	70
	Sample line temp. (°C)	100	100	150	150
	Transfer line temp. (°C)	120	120	160	160
	Equilibrating time (min)	2,00	5,00	5,00	5,00
	Pressurizing time (min)	0,10	0,10	0,50	0,50
	Pressure equilib. time (min)	0,10	0,10	0,10	0,10
	Load time (min)	0,50	0,50	0,10	0,10
	Load equilib. time (min)	0,10	0,10	0,10	0,10
	Injection time (min)	1,00	1,00	1,00	0,50
	Needle flush time (min)	0,50	0,50	2,00	16,00
	GC cycle time (min)	38,0	45	34	34
GC-2030	Column oven temp. (°C)	40	40	40	40
	Pressure (kPa)	91,7	44,0	91,0	91,0
	Total flow (ml/min)	12,30	8,30	16,60	16,60
	Column flow (ml/min)	1,64	0,93	1,62	1,62
	Purge flow (ml/min)	3,00	3,00	15,00	15,00
	Oven temp. program	40 °C (hold 4 min) 8 °C/min to 60 °C 5 °C/min to 85 °C (hold 2 min) 30 °C/min to 220 °C (hold 2 min)	40 °C (hold 2 min) 10 °C/min to 100 °C 1 °C/min to 120 °C 10 °C/min to 200 °C	40 °C (hold 2 min) 20 °C/min to 105 °C 5 °C/min to 140 °C 20 °C/min to 230 °C (hold 8 min)	40 °C (hold 2 min) 20 °C/min to 105 °C 5 °C/min to 140 °C 20 °C/min to 230 °C (hold 8 min)
	HS pressure (kPa)	44	44	76	76
GCMS-QP2020 NX	Start time (min)	1,10	2,10	2,10	2,10
	End time (min)	20,0	36,0	20,5	20,5
	M/z range (m/z)	40-500	40-200	50-200	50-200

Appendix 2. Preparation of solutions and standards

The initial amount of substance of 99% furfural, when assumed 100%, was calculated based on the properties of the compound (Table 1) using the equation 1.

$$n = \frac{m}{M} = \frac{1160\ g}{96{,}084 \frac{g}{mol}} = 12{,}0727697\ mol \approx 12{,}07\ \mathrm{mol} \tag{1}$$

where n is the amount of substance, m is the mass (g/l) derived from density and M is the molar mass of the compound.

Furthermore, the molar concentration of the 99% furfural per one litre was calculated applying the equation (2) as follows:

$$M = \frac{n}{V} = \frac{12{,}07\ mol}{1\ l} = 12{,}07\ mol/l \tag{2}$$

where M is the concentration (mol/l), n is the amount of substance and V is the volume (l).

To prepare stock solutions and standards, equation (3) was applied

$$C_1 V_1 = C_2 V_2 \tag{3}$$

where C1 is the initial concentration (mol/l), V1 is the initial volume, C2 is final concentration and V2 the final volume of the solution. Therefore, the volume of the original solution required for the desired concentration can be calculated using the equation (4)

$$V_1 = \frac{C_2 V_2}{C_1} \tag{4}$$

where V1 is the pipetted volume, C2 is the final concentration, V2 is the final volume and C1 is the initial concentration.

Appendix 3. Some compounds detected from wastewater samples (1-2)

TABLE 6. Sample 1.

Peak #	R. time	Area	Name
1	2,294	1595699	Pentanoic acid, 3-methyl-4-oxo-
2	2,807	1274084	Trimethylsilyl fluoride
3	3,846	689064	2-Fluoropropene
4	4,463	461309	1-Deoxy-2,4-O,O-methylene-d-xylitol
5	5,330	256007	Cyclopentanone
6	5,584	10600618	1-Butanol, 3-methyl-
7	6,293	1276523	Furfuryl ethyl ether
8	7,087	191909	Formic acid, hexyl ester
9	8,334	16674137	Acetic acid
10	8,573	93581	Furfural
11	9,229	715933	Ethanone, 1-(2-furanyl)-
12	9,658	231999	2-Furanmethanol, acetate
13	11,266	2990929	Butanoic acid
14	11,867	70065788	3-Furanmethanol
15	14,597	728699	Mequinol

TABLE 7. Sample 2.

Peak #	R. time	Area	Name
1	2,289	2174315	Pentanoic acid, 3-methyl-4-oxo-
2	2,793	933837	Perfluoropropionic acid, TMS derivative
3	4,458	476994	1-Deoxy-2,4-O,O-methylene-d-xylitol
4	5,328	749829	Cyclopentanone
5	5,583	11695187	1-Butanol, 3-methyl-
6	6,289	1310265	Furfuryl ethyl ether
7	6,563	417809	Cyclopentanol
8	8,362	1459229	Acetic acid
9	8,569	565151	Furfural
10	9,225	724405	Ethanone, 1-(2-furanyl)-
11	11,265	1764126	Butanoic acid
12	11,865	64430473	3-Furanmethanol
13	14,596	826806	Mequinol
14	15,112	2480846	Phenylethyl Alcohol
15	17,731	464894	Ethyl 9-hexadecenoate

Appendix 4. Some compounds detected from wastewater samples (3)

TABLE 8. Sample 3.

Peak #	R. time	Area	Name
1	2,280	843943	Pentanoic acid, 3-methyl-4-oxo-
2	2,793	771811	Perfluoropropionic acid, TMS derivative
3	2,883	203043	Furan, 2,5-dimethyl-
4	3,843	357106	2-Fluoropropene
5	4,453	265268	1-Deoxy-2,4-O,O-methylene-d-xylitol
6	5,545	6639545	1-Pentanol
7	6,294	856927	Furfuryl ethyl ether
8	8,320	10559715	Acetic acid
9	8,563	132364	Furfural
10	8,739	230373	Oxalic acid, 2TMS derivative
11	9,220	324024	Ethanone, 1-(2-furanyl)-
12	11,255	2747874	Butanoic acid
13	11,857	34279610	3-Furanmethanol
14	14,600	262076	Mequinol
15	15,116	911571	Phenylethyl Alcohol