Short application note

Food & beverage

5-Hydroxymethylfurfural in biomass

Keywords: ALEXYS™ Carbohydrate Analyzer, SweetSep™ AEX20, HPAEC-PAD, biomass, pulp, 5-hydroxy-methylfurfural, HMF

Introduction

5-Hydroxymethylfurfural (HMF) is a chemical derived from biomass, offering a sustainable raw material that can be converted into valuable compounds like 5-ethoxymethyl-furfural (EMF) and 2,5-furandicarboxylic acid (FDCA), which are precursors to pharmaceuticals, polymers, resins, and biofuels [1,2]. It is typically produced from carbohydrate-rich biomass such as cellulose and starch through a three-step process: (a) hydrolysis of glucan into glucose, (b) isomerization of glucose into fructose, and (c) dehydration of fructose to form HMF.

Accurate detection and quantification of HMF are important in biomass utilization. There are two main motivations for monitoring HMF levels: (1) quantification of HMF in biomass conversion processes where HMF is the target product, enabling yield optimization [1,2], and (2) to control the concentration in applications where HMF is an undesirable side product, such as in the bioethanol production. HMF is known as a fermentation inhibitor, reducing ethanol yields [3,4].

Various methods are available for the quantification of HMF, for example based on UV spectrometry, HPLC-UV/DAD and GC-MS [5,6] .UV- spectrophotometry measurements of HMF are typically based on its UV absorbance at 284 nm [5]. Although, it is simple and fast the specificity of the method is low which might lead to inaccurate quantification due to

Fig. 2. ALEXYS Carbohydrate Analyzer.

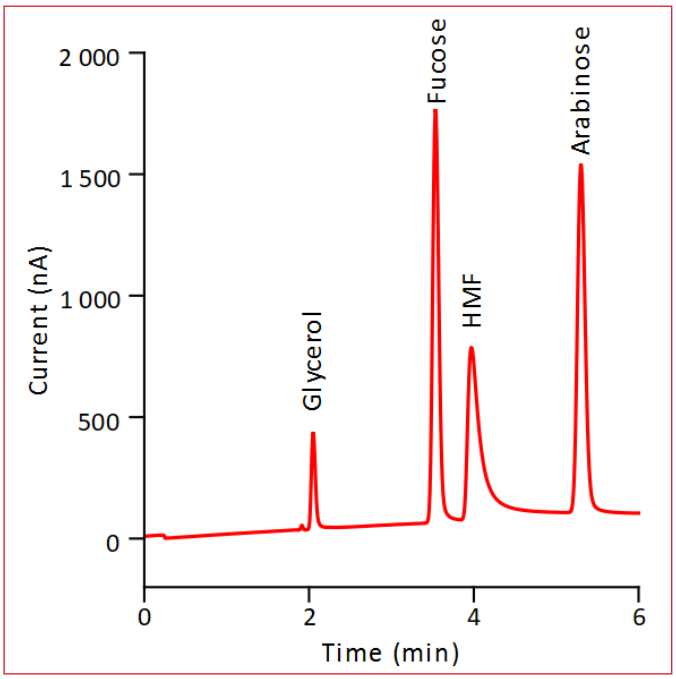

Fig. 1. Chromatogram obtained from a 3 µL injection of 10 µg/mL glycerol, fucose, HMF and, arabinose in DI water. Separation and detection was achieved using the HPAEC-PAD conditions and gradient program shown in Table 1 and 2, respectively. The total run time is 22 minutes including wash/regeneration and equilibration step.

Table 1. HPAEC-PAD conditions

HPLC	ALEXYS™ Carbohydrate Analyzer (Antec Scientific)
Columns	SweetSep™AEX20, 2.1 x 50 mm precolumn, 5µm SweetSep™AEX20, 2.1 x 200 mm column, 5µm Borate ion trap, 2.1 x 50 mm column, 10 µm (all columns Antec Scientific)
Mobile phase	A: DI water B: 100 mM NaOH C: 100 mM NaOH + 100 mM NaOAc Eluents blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min
Backpressure	About 170 bar
Injection volume	3 µL
Temperature	4°C for sample cooling (AS6.1L), 25°C for separation (CT2.1) and 45°C for detection (DECADE Elite)
Flow cell	SenCell Au WE, HyREF Pd RE, AST setting 1
Potential waveform (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
Range	5 µA/V
I-cell	About 0 - 0.2 µA
ADF	0.5 Hz

interference with other compounds. Methods based on HPLC combined with UV/DAD detection have good selectivity but are less sensitive compared to for example MS or electrochemical detection. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) offers high selectivity and sensitivity and is an attractive alternative for the analysis of HMF in complex biomass matrices.

In this application note fast high-resolution separation and sensitive detection of HMF in biomass is demonstrated, using the new Antec Scientific carbohydrate analyzer in combination with the SweetSep™ AEX20 column. The AEX20 is a novel anion -exchange stationary phase based on a highly mono-disperse 5 µm ethylvinylbenzene divinylbenzene copolymer (80% crosslinked) substrate particles coated with functionalized nanobeads containing dual ion exchange sites (quaternary amine and tertiary amine). The fast separation and high resolution of the column is evident from Figure 1, whereby HMF elutes in less than 5 minutes without interference from other compounds.

Method

The HPAEC-PAD conditions and gradient program are listed in Table 1 and 2, respectively. The analysis was performed using the ALEXYS Carbohydrate analyzer (Fig. 2), which is a dedicated HPAEC-PAD system with a metal-free flow path, optimized for the sensitive analysis of carbohydrates. The system consists of the ET210 eluent tray, a P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, DECADE Elite electrochemical detector and SenCell flow cell. The ET210 eluent tray has an integrated gas distribution system to blanket the headspace of the eluent bottles with inert gas (He or N_2) to avoid diffusion of CO_2 into the eluents and minimize the formation of carbonate ions, ensuring reproducible analysis. A 2.1 x 200 mm AEX20 analytical column in combination with a 2.1 x 50 mm AEX20 precolumn was used for the analysis. The 2.1 mm ID microbore columns are operated at a low flow rate of 0.18 mL/min, minimizing the mobile phase consumption and waste, thus reducing the environmental impact.

Table 2. Gradient program

Time (min)	*Mobile phase*	*%A*	*%B*	*%C*	*Description*
0.00	10 mM NaOH	90	10	0	Gradient elution &
2.00	15 mM NaOH	50	50	0	detection
2—7	100 mM NaOH, 100 mM NaOAc	0	0	100	column clean-up/ regeneration
7—22	10 mM NaOH	90	10	0	Equilibration to starting conditions

As a precaution a 2.1 x 50 mm borate ion trap was installed in the solvent line between pump and injector to eliminate the presence of borate contaminants in the mobile phase. Borate ions can form complexes with some carbohydrates causing peak tailing and thus loss of peak symmetry. A temperature of 25 °C was selected for optimal separation of HMF from the sugars in the sample using the AEX20. The detection temperature was set to 45°C to assure optimum detection sensitivity.

Sample preparation

The biomass hydrolyzate samples, dissolved pulp (DP) and paper grade pulp (PGP), were kindly provided by the Thüringisches Institut für Textil- und Kunststoff-Forschung (TITK, Rudolstadt, Germany). The DP and PGP sample (100 mg) were prepared by hydrolysis in 0.25 M sulfuric acid and filtered over a 0.45 µm nylon filter. Subsequently, the filtered hydrolyzate samples were neutralized, diluted 10 times and filtered through a 0.22 µm polyethersulfone filter (25 mm Ø FFL/MLS) prior to injection.

Results

An example chromatogram obtained with the 3 µL injection of a 10 µg/mL standard mix is depicted in Figure 1. The chromatogram shows baseline separation (Rs > 1.5) of HMF along with 3 other components which were all eluted within 6 minutes. The use of a NaOH gradient results in a slight baseline drift, caused by the change in pH and mobile phase composition. The peak efficiency for HMF is approximately 14,000 theoretical plates. Noticeable peak tailing is observed for HMF, with a tailing factor of 3.6.

The LOD was calculated as the analyte response corresponding to 3× the ASTM noise (average peak-to-peak baseline noise of 4 segments of 0.5 min). The noise was determined using a 2-minute section of the baseline of a blank injection between t = 2.5 min to 4.5 min. The peak height response obtained from a 3 µL injection of a 0.1 µg/mL standard was used to calculate the LOD. A detection limits of 10 ng/mL (corresponding to 79 nmol/L) was obtained for HMF, demonstrating the high sensitivity of the HPAEC-PAD method. The method's repeatability was evaluated by 10 consecutive injections of a 10 and 1 µg/mL standard in DI water. Excellent repeatability was achieved with RSD values for peak area and retention time of < 0.28 % and < 0.24%, respectively. The linearity of the method was assessed over a large concentration range of 0.1– 50 µg/mL (5 calibration points) for HMF. A quadratic fitting was applied ignoring the origin and using a weighted factor of $1/concentration^2$. A relative standard error of 1.99% was obtained for HMF, demonstrating high accuracy of the calibration curve.

Sample analysis

The chromatograms of the DP & PGP sample are shown in Figure 3 and 4. Both samples were 10× diluted before injection. To ensure correct peak identification, the samples were spiked with the standard mix resulting in a final concentration of 0.5 µg/mL of the analytes in the spiked sample. The HMF concentration (based on the calibration curves) in the DP sample was estimated to be 30.4 µg/mL. The HMF concentration in the PGP sample was found to be 20.1 µg/mL. The detected concentrations are low, and for example, well below the reported inhibition threshold for ethanol production from biomass hydrolyzates, which occurs at approximately 300 µg/mL HMF [4].

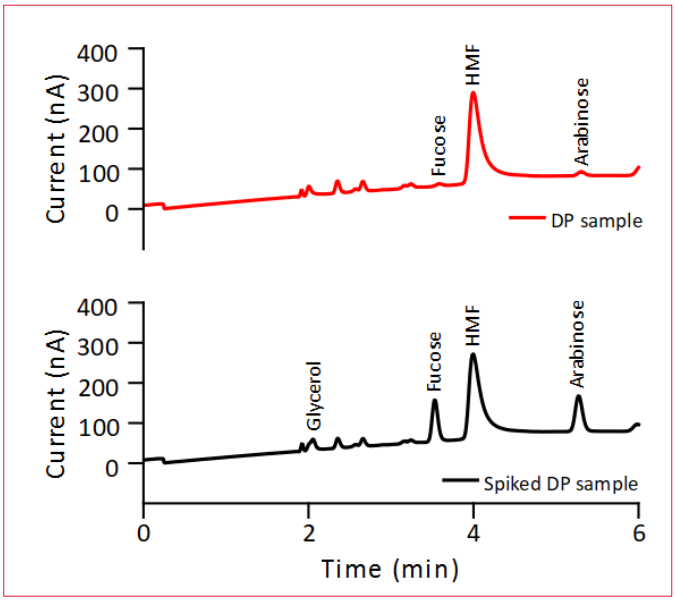

Fig. 3. Overlay of chromatograms obtained from 3 µL injections of the DP sample spiked with 0.5 µg/mL standards (black) and the DP sample (red).

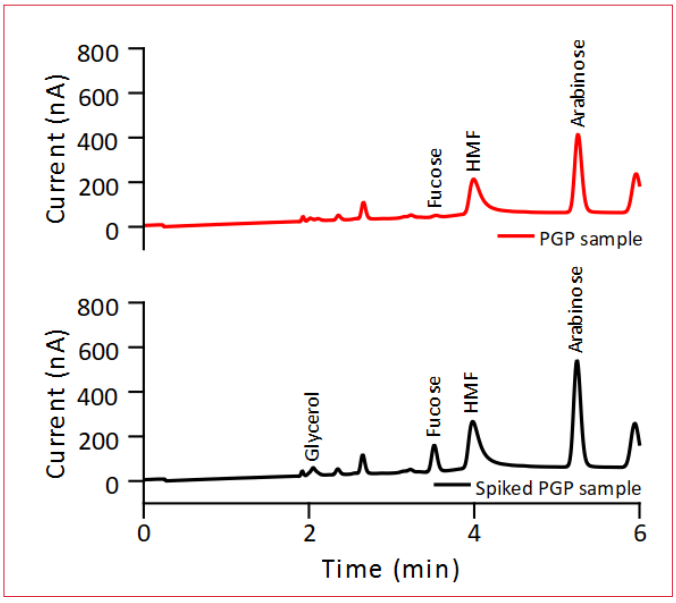

Fig. 4. Overlay of chromatograms obtained from 3 µL injections of the PGP sample spiked with 0.5 µg/mL standards (black) and the PGP sample (red).

Analysis of the neutral sugar composition by HPAEC-PAD, published in application note 220_037, had shown that both pulp samples contained predominantly glucose, with minor amounts of fructose. The small amounts of HMF found in the samples can be likely explained by partial conversion of fructose into HMF during acid hydrolysis. Under these conditions the formation of HMF from glucose, by isomerization to fructose followed by dehydration into HMF, is expected to be neglectable [8].

In conclusion, the ALEXYS Carbohydrate Analyzer with the SweetSep™ AEX20 column enables rapid, high-resolution separation (within 5 minutes) in combination with sensitive detection of HMF in biomass hydrolyzates.

References

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Ordering information

ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Columns	
260.0021	SweetSep™ AEX20, 2.1 x 200 mm column, 5 µm
260.0026	SweetSep™ AEX20, 2.1 x 50 mm precolumn, 5 µm
260.0031	Borate ion trap, 2.1 x 50 mm column, 10 µm
Software*	
195.0035	Clarity CDS single instr. incl. LC, AS module

*) The ALEXYS Carbohydrate Analyzer can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS and Agilent OpenLab CDS. Please contact Antec Scientific for more details.

Reagents, standards and sample prep accessories

NaOH (50% w/w/Certified)	Fisher Scientific, pn SS254-500
Sodium acetate (NaOAc), 100%	Sigma Aldrich, pn 79714
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
5-Hydroxymethylfurfural (HMF)	Biosynth, pn FH10853
Fucose	Sigma Aldrich, pn F2252-5G
Glycerin	De Tuinen Natural Care, pn 6100000734
Arabinose	Sigma Aldrich, pn A3131
Syringe filter	0.22 µm PES (Polyethersulfone) 25 mm Ø FFL/MLS
Nitrogen 5.0 (purity 99.999%)	Messer Netherlands, pn 100542102

For research purpose only. The information shown in this short application note is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control and may be adjusted accordingly. Specifications mentioned are subject to change without further notice.

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