Short application note

Food & beverage

5-Hydroxymethylfurfural in honey

Keywords

ALEXYS carbohydrate analyzer, HPAEC-PAD, SweetSep™ AEX20, honey, 5-hydroxymethylfurfural, HMF, quality assurance, authenticity verification

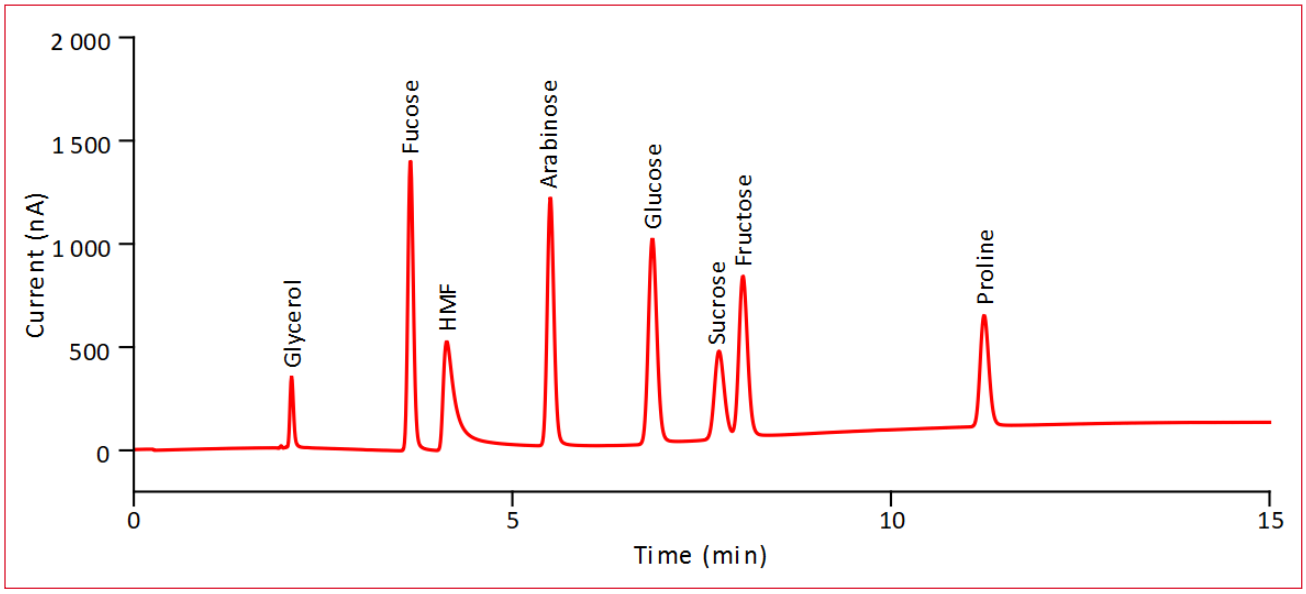

Fig. 1. Chromatogram obtained from a 3 µL injection of 10 µg/mL glycerol, fucose, HMF, arabinose, glucose, sucrose, fructose and proline in DI water. Separation and detection were achieved using the HPAEC-PAD conditions and gradient program shown in Table 1 and 2, respectively. The total run time is 35 minutes including wash/regeneration and equilibration step.

Introduction

Honey is a natural sweetener that is valued for its health benefits, and diverse uses in food and medicine. However, its quality can degrade over time or due to improper processing or storage. A key indicator of honey's quality is the level of 5-hydroxymethylfurfural (HMF), a compound formed due to the degradation of sugars. Elevated HMF levels are indicative of thermal processing or aging, and are therefore used to assess

Fig. 2. ALEXYS Carbohydrate Analyzer.

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 180 bar
Injection volume	3 µL
Temperature	4°C for sample cooling (AS6.1L), 25°C for separation (CT2.1), 35°C for detection (DECADE Elite)
Flow cell	SenCell Au WE, HyREF Pd RE, AST setting 2
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—0.2 µA
ADF	0.5 Hz

freshness and authenticity [1]. Monitoring HMF levels also ensures compliance with food safety standards (EU Directive 2001/110/EC & Codex Alimentarius) and helps protect consumers from adulterated or poor-quality honey [2,3].

High-performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is a powerful technique for sensitive analysis of sugars and degradation products such as HMF in honey. In this application note we demonstrate a fast, high-resolution separation of HMF both in untreated honey and thermally stressed honey, using the new Antec Scientific SweetSep™ AEX20 column. The SweetSep AEX20 is an anion-exchange stationary phase based on a highly monodisperse 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 resolving power of the column is evident from figure 1, where HMF elutes around 4.1 minutes without interference from other sugars and amino acids.

Method

The setup & conditions of the method and gradient program are listed in table 1 and 2, respectively. The analysis was performed using the ALEXYS Carbohydrate Analyzer (Figure 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 an ET210 eluent tray, P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, DECADE Elite electrochemical detector and a SenCell flow cell. The ET210 eluent tray has an integrated gas distribution system to blanket the headspace of the eluent bottles with inert gas (Helium or Nitrogen) 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 were 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.

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.

Sample preparation

A honey sample was prepared by dissolving 1.0 gram in 100 mL of deionized water followed by sonication of the solution for 10 minutes. Additionally, 0.5 g of the same honey was weighed into a beaker and incubated in a 90 °C water bath for 6 hours to produce a thermally stressed sample. Subsequently, 0.1 gram of the thermally stressed sample was dissolved in 10 mL deionized water. Both untreated honey and thermally stressed honey samples were filtered using a 0.22 µm polyethersulfone (PES) filter and diluted in two-fold prior to injection.

Table 2. Gradient program

Time (min)	*Mobile phase*	*%A*	*%B*	*%C*	*Description*
0.00	10 mM NaOH	90	10	0	Gradient elution & detection
15.00	50 mM NaOH	50	50	0	
15—20	100 mM NaOH, 100 mM NaOAc	0	0	100	column clean-up/ regeneration
20—35	10 mM NaOH	90	10	0	Equilibration to starting conditions

Results

An example chromatogram of the standard mix containing HMF and several sugars is shown in Figure 1. HMF elutes at approximately 4.1 minutes, and all potential interfering compounds were baseline separated, with a resolution (Rs) greater than 2. The peak efficiency for HMF is about 15,000 theoretical plates. A noticeable peak tailing is observed for HMF, with a tailing factor of 3.6.

The limit of detection (LOD) was calculated as the analyte response corresponding to 3× the ASTM noise (average peak-to-peak baseline noise of 10 segments of 0.5 min). The noise was determined using a 5-minute section of the baseline of a blank injection between t = 10 min to 15 min. The peak height obtained from a 3 µL injection of a 0.1 µg/mL standard was used to calculate the LOD. A detection limits of 181 nmol/L (corresponds with 23 ng/mL) was obtained for HMF, demonstrating the high sensitivity of the presented HPAEC-PAD method. The repeatability of the method was evaluated by 10 consecutive injections of both 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 < 0.14%, respectively. The linearity of the method was investigated over a 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 2.45% was obtained for HMF, demonstrating the high accuracy of the calibration curve.

Sample analysis

The overlay of the chromatograms of the honey sample and the thermally stressed honey sample is presented in Figure 3. The HMF concentration is determined to be 15.2 mg/kg in the honey sample. According to the EU Directive and the Codex Alimentarius, the maximum permittable amount of HMF is 40 mg/kg for honey produced under European conditions and 80 mg/kg for honey produced from tropical countries [2,3]. Usually a small amount of HMF is naturally present in fresh honey [4]. In contrast, the thermally stressed honey sample contains a significantly higher HMF concentration (167.9 mg/kg of honey), exceeding the limits set in the EU Directive and the Codex Alimentarius for HMF in honey. To assess the repeatability, both samples were analyzed in duplicate. The honey sample showed a difference of only 0.05 mg/kg between duplicates, and the thermally stressed honey sample showed a difference of 0.5 mg/kg. These values are well within the acceptable repeatability limits defined by the International Honey Commission [5].

Overall, the presented method enables fast, high-resolution separation and sensitive quantification of HMF in honey within 5 minutes using the ALEXYS carbohydrate analyzer in combination with the new SweetSep™ AEX20 column.

References

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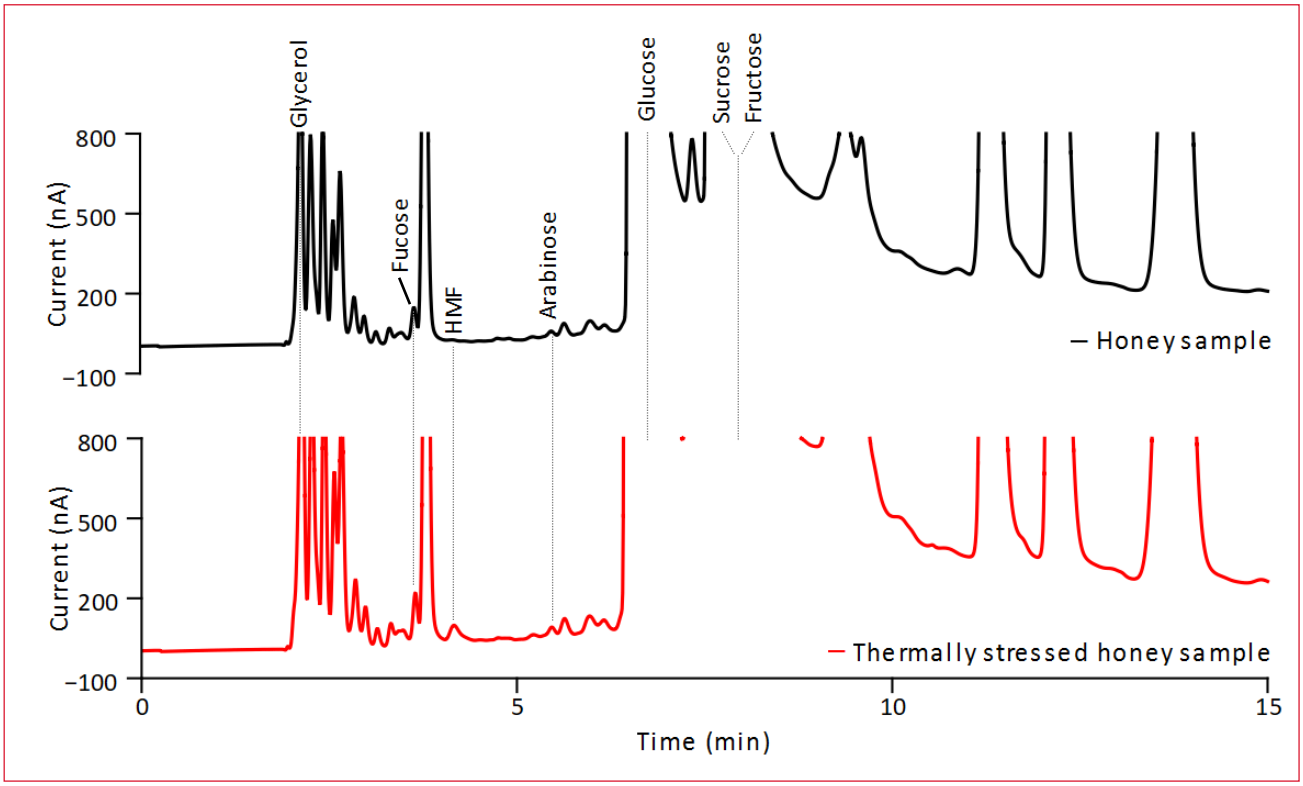

Fig. 3. Overlay of chromatograms obtained from 3 µL injections of honey sample (black curve), and a thermally stressed honey sample (red curve).

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. 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
Fructose	Sigma Aldrich, pn F0127
Glucose	Sigma Aldrich, pn G8270
Sucrose	Sigma Aldrich, pn S9378
Fucose	Sigma Aldrich, pn F2252-5G
L-proline	Sigma Aldrich, pn P0380
100% 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

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