

Sensitive Determination of Hydroxymethylfurfural in Honey

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Introduction

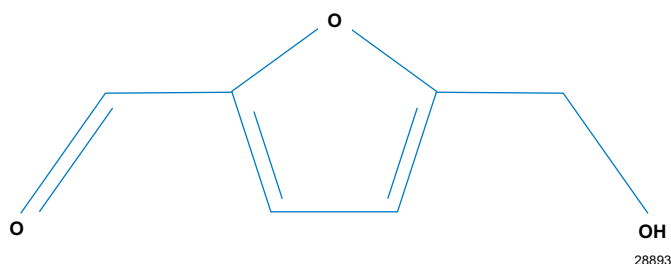
Hydroxymethylfurfural (HMF) is a water-soluble heterocyclic organic compound derived from sugars. It is a furan derivative with both aldehyde and alcohol functional groups (Figure 1). Very low amounts of this compound are found naturally in fresh sugar-containing foods including milk, honey, fruit juices, spirits, and bread. Additionally, HMF is produced during food pasteurization and cooking as a result of dehydration of sugars such as glucose and fructose¹ and in the initial stages of the Maillard reaction,² a reaction between sugars and proteins responsible for changes in color and flavor of food. HMF is also formed during extended food storage under acidic conditions that favor its generation. Therefore, it is an indicator of excessive heat treatment, spoilage, and of possible adulteration with other sugars or syrups.

The National Institute of Environmental Health Sciences nominated HMF for toxicity testing³ based on the potential for widespread exposure through consumed foods, and evidence for carcinogenic potential of other members of this class. As a result, many countries impose restrictions on maximum levels of HMF in food and beverages.⁴

There are current spectrophotometric and HPLC methods for HMF determination. One commonly used method is based on spectral absorbance at 284 nm.^{5,6} This direct absorbance measurement may be subject to interferences from other compounds present in complex matrices. In the HPLC method, HMF is separated using a reversed-phase column, with water and methanol as the mobile phase, and detection by UV absorbance.⁷

This work describes a High Performance Anion Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) based method for the determination of HMF in honey, pancake syrup, and fructose solution.

FIGURE 1. Hydroxymethylfurfural



An ion chromatography system, a Thermo Scientific Dionex CarboPac™ PA1 column, electrolytically generated hydroxide eluent, and electrochemical detection with disposable Au on polytetrafluoroethylene (PTFE) working electrodes were used. The Dionex CarboPac PA1 is a high-capacity rugged column suitable for determining mono- and disaccharides, and provides high resolution for HMF in a wide variety of matrices. The data presented here demonstrates the linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, and recovery of HMF in diverse matrices. PAD is shown to be an appropriate detection technique for HMF, with a broad linear range and low detection limits. Disposable electrodes provide short equilibration time and greater electrode-to-electrode reproducibility compared to conventional electrodes. Compared to other disposable Au electrodes, the Au on PTFE electrodes demonstrated longer lifetime and the ability to operate at higher hydroxide concentrations. The method shown provides good sensitivity, consistent response, and can be used routinely for HMF analysis.

Experimental Details

Thermo Scientific Dionex ICS-3000 or ICS-5000 Ion Chromatography system including:

Gradient or Isocratic Pump, with vacuum degas option
DC Detector/Chromatography Module
10 µL Injection Loop
Electrochemical Detector
Carbohydrate PTFE Disposable Au Working Electrodes
Ag/AgCl Reference Electrode
3 mil PTFE Gaskets
AS Autosampler
Thermo Scientific Dionex Chromeleon™ Chromatography Data System Software

Method

Columns: Dionex CarboPac™ PA1 Analytical, 4 × 250 mm,
Dionex CarboPac™ PA1 Guard, 4 × 50 mm
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL (full loop)
Temperature: 30 °C
Back Pressure: 2400 psi
Eluent: 50 mM KOH
Eluent Source: EGC II KOH with CR-ATC
Detection: PAD
Background: 30–70 nC
Working Electrode: Carbohydrate PTFE Disposable Au working Electrodes
Reference Electrode: Ag/AgCl mode
Noise: 30 pC
Carbohydrate Waveform

Sample Preparation

Honey and Syrup

Honey and syrup samples were prepared by dissolving 1 g in 100 mL of DI water and sonicating for 10 min. Syrup samples were further diluted 2-fold with DI water before injection.

Fructose

A stock solution of fructose was prepared by dissolving 100 mg in 100 mL DI water. The solution was diluted 500-fold with DI water before injection. The thermally stressed honey, syrup, and fructose solutions were prepared by heating samples at 100 ± 10 °C for 4 h, then cooling to room temperature before dilution and injection.

Precautions

As the honey and syrup samples contain high concentrations of sugars such as glucose and sucrose, carryover may be observed. A syringe flush of 1000 µL is recommended between samples. Washing the column with 100 mM KOH is recommended if retention time shifting is observed. The application of 100 mM KOH changes the system equilibrium; re-equilibration using 50 mM KOH for ~2 h is recommended to achieve high precision.⁸

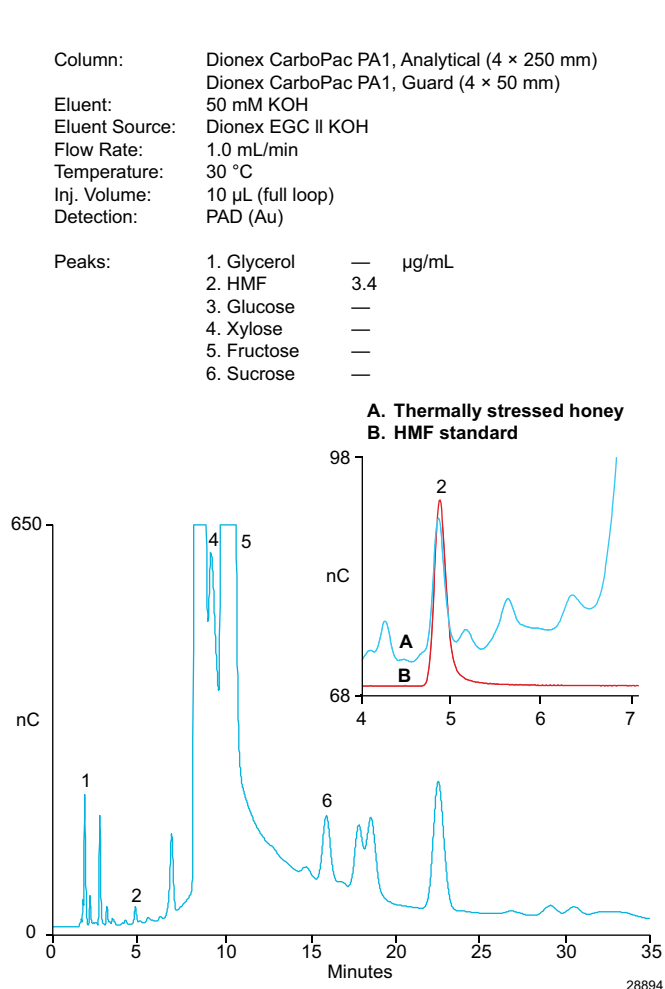
Results

Figure 2 shows determination of HMF in a thermally stressed honey sample. HMF elutes at 4.8 min, and can be detected without interference from the other sugars. The HMF content in this sample was determined to be 330 mg/kg of honey. Typically, fresh honey has low amounts of HMF (<15 mg/kg). The HMF concentration increases as honey undergoes heat treatment to prevent crystallization and reduce viscosity to facilitate filling.⁹ The EU Directive (110/2001) and the codex alimentarius (Alinorm 01/2000) standards limit HMF to 40 mg/kg for honey produced in Europe and 80 mg/kg for honey coming from tropical countries.⁴ HMF in the fresh honey sample was determined to be 0.17 µg/mL (equal to 17 mg HMF/kg of honey, Table 1).

Table 1: Intra- and Between-Day Precisions for Honey

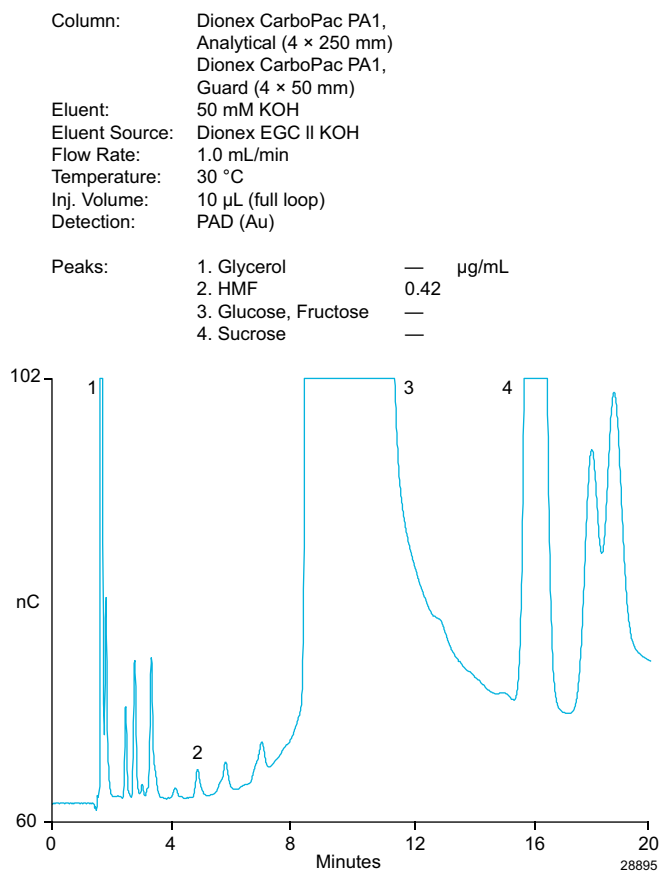
Sample	Amount (µg/mL)	RT Precision (RSD)		Peak Area Precision (RSD)	
		Intra Day	Between-Day	Intra Day	Between-Day
Fresh Honey	0.17				
Thermally Stressed Honey	3.4	0.09	0.06	1.19	1.17

FIGURE 2. HMF in thermally stressed honey.



The chromatogram of thermally stressed pancake syrup (Figure 3) shows the separation of HMF from other thermal degradation products. HMF is a product of thermal degradation of fructose—the main constituent of pancake syrup. HMF in high-fructose corn syrup (HFCS) is also a problem for beekeepers, as they use HFCS as a source of sugar to feed bees when natural nectar sources are limited. Note that complex matrices like pancake syrup may exhibit later eluting peaks (e.g. pancake syrup has a peak at ~55 min, not shown). This long retention time may interfere with subsequent injections if a shorter run time is used.

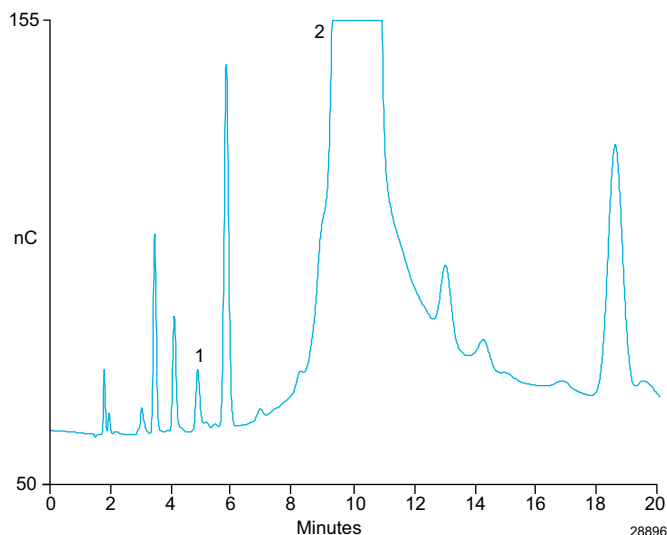
FIGURE 3. HMF in pancake syrup (high fructose corn syrup).



The HPAE-PAD method described here was used for HMF detection in fructose. The United States Pharmacopeia (USP)¹⁰ and Food Chemicals Codex (FCC)¹¹ have monographs for determination of HMF in fructose and fructose injections. The USP monograph is a spectrophotometric method based on UV absorbance of HMF at 283 nm. HMF is formed during sterilization and storage and is present as an organic impurity in fructose; it must be quantified and meet USP requirements before use as a food substance or as infusion fluids. The chromatogram of thermally stressed fructose solution (Figure 4) shows the separation of HMF from other thermal degradation products. HMF is also formed in aqueous dextrose solutions and an HPAE-PAD-based method has been reported for quantification of HMF in commercial dextrose solutions.

FIGURE 4. HMF in thermally stressed fructose solution.

Column: Dionex CarboPac PA1, Analytical (4 × 250 mm)
 Dionex CarboPac PA1, Guard (4 × 50 mm)
 Eluent: 50 mM KOH
 Eluent Source: Dionex EGC II KOH
 Flow Rate: 1.0 mL/min
 Temperature: 30 °C
 Inj. Volume: 10 µL (full loop)
 Detection: PAD (Au)
 Peaks:
 1. HMF 2.1 µg/mL
 2. Fructose —



Linear Range, Limit of Quantitation, Limit of Detection

To determine linearity of the method, calibration standards were injected in triplicate covering the expected range of HMF in food samples. Calibration plots produced a coefficient of determination value of 0.9998 in the range 0.1-50 µg/mL (Table 2). A least squares regression fit with weighting was used to accurately represent the lower values of the calibration curve. Typical baseline noise for this method was 20-40 pC. The LOD and LOQ for this method were 0.04 µg/mL and 0.10 µg/mL, respectively.

Precision

The peak area and retention time precisions were determined for seven replicate injections of a HMF standard (Table 1). The retention time precision (RSD) was 0.1, and the peak area precision was 0.4. The high retention time precision is attributable to consistent generation of high-purity KOH using the eluent generator. Intraday and between-day precision for HMF in honey was evaluated over three consecutive days. The RT and peak area precisions are summarized in Table 2. The RT precision range was 0.06-0.09%, and peak area precisions were 0.7%. The high precision results indicate this method is ideal for use with complex matrices.

Accuracy

The accuracy of the method was verified by determining recoveries of HMF in spiked honey over three consecutive days. The amount of HMF in a fresh honey sample was 0.17 µg/mL (Table 2; this equals 17 mg of HMF per kg of honey). The thermally stressed honey had 3.4 µg/mL HMF (Figure 2), and was spiked with 2.9 µg/mL. Intra-day concentration RSD was 1.7% for honey. The average recovery of HMF in honey was 103% (Table 3).

Table 2: Linear Range and Precisions for HMF Standards

Analyte	Sample (Range µg/mL)	Coeff. of Determ. (r ²)	RT (min)	Concentration Used for Precision Injections (µg/mL)	RT Precision (RSD)	Peak Area (nC* min)	Peak Area Precision (RSD)
HMF	Honey (0.1-50)	0.9998	4.85	0.5	0.12	0.55	0.50

Table 3: Recoveries of HMF in Spiked Honey

Analyte	Sample	Amount Found (µg/mL)	Amount Added (µg/mL)	Average Recovery (n = 3 days)
HMF	Thermally Stressed Honey	6.4	2.9	102.6

Conclusion

- An accurate, reliable HPAE-PAD method for HMF analysis is shown which provides good linear range, high precision, low detection limits, that can be used for online monitoring of HMF levels in food applications.
- The disposable gold working electrode provides consistently high detector response, assuring greater instrument-to-instrument and lab-to-lab reproducibility.
- This configuration needs only addition of DI water for continuous operation.

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