

Determination of hydroxymethylfurfural in honey according to DIN 10751 (part 3, february 2002)

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Abstract

This application note describes the determination of hydroxymethylfurfural in honey using HPLC-UV.

Introduction

Hydroxymethylfurfural (HMF), also 5-(hydroxymethyl)furfural, occurs naturally in most honeys and can be used as an indicator for heat-treatment or for overlong storage time at elevated temperatures [1]. It is formed by the dehydration of certain sugars [2]. The content of HMF in honey is regulated by food law, for instance in Germany the Regulation for honey sets the limit for fresh honey at 40 mg/kg. An exception is tropical honey which must contain less than 80 mg/kg HMF [3]. Processed honey for baking can also have higher amounts of HMF. Therefore HMF is an important parameter of food control. The determination of HMF in honey is described in DIN 10751 (part 3, february 2002) [4]. The BVL has published this procedure used by German Federal States control authorities for analyzing food and feed in the official collection of test methods under part L. 40.00-10/3 (§ 64 LFGB) [5].

The determination of HMF is performed by measuring the absorbance of a clarified aqueous honey solution against a reference solution by RP-HPLC-UV at 284 nm. This application note describes the determination of HMF of two honey samples according to the requirements of food law.

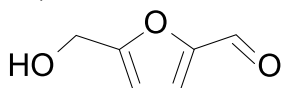


Figure 1: Compound of interest.

Sample pretreatment

- Homogenize honey sample by stirring
- Weigh 10 g sample into a beaker
- Add 50 mL water and dissolve sample completely
- Fill sample solution in a volumetric flask (100 mL)
- Add clearing reagents* Carrez I and Carrez II
- Fill up to 100 mL with water
- Centrifuge at room temperature at 13500 rpm for 5 min
- Filter upper layer through CHROMAFIL® PET-20/13 (REF 729222)

* Clearing reagents:

Carrez I: Solve 15.0 g potassium ferrocyanide (II) trihydrate in water

Carrez II: Solve 30.0 g zinc acetate dihydrate in water

Subsequent analysis: HPLC-UV

Chromatographic conditions

Column:

EC 100/3 NUCLEODUR® C₁₈ Gravity, 3 µm (REF 760084.30)

Eluent A:

10 mmol/L disodium phosphate in water, adjusted at pH 3.0 with phosphoric acid

Eluent B:

methanol

Gradient:

hold 5 % B for 7.5 min, in 5 min to 80 % B, hold 80 % B for 7.5 min, back to 5 % B in 0.1 min and hold for 5.0 min

Flow rate:

1.0 mL/min

Temperature:

25 °C

Injection volume:

10 µL

Detection:

UV: 284 nm

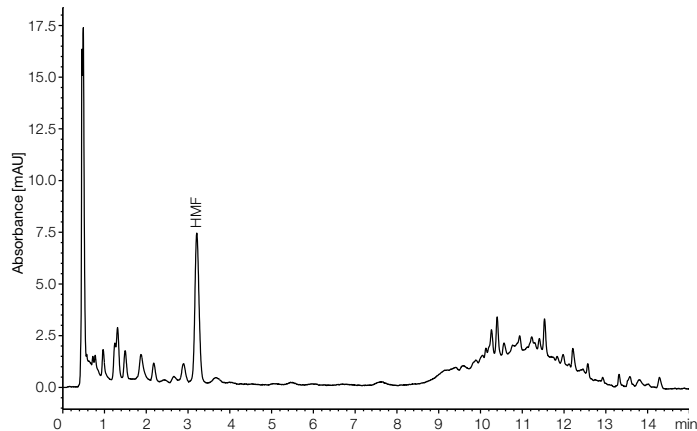


Hydroxymethylfurfural in honey

Chromatograms

A:

Wildflower



Sunflower

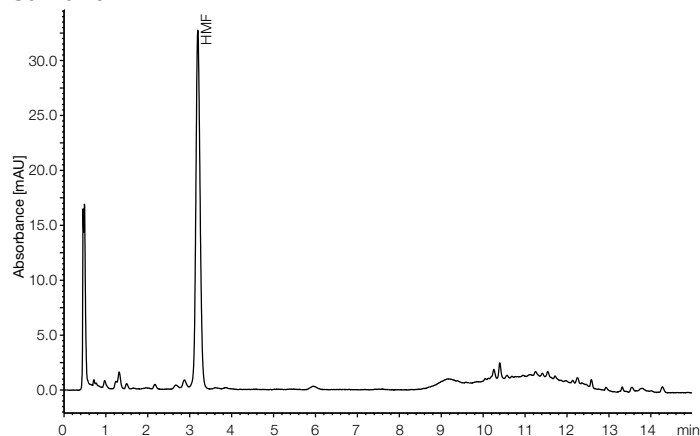
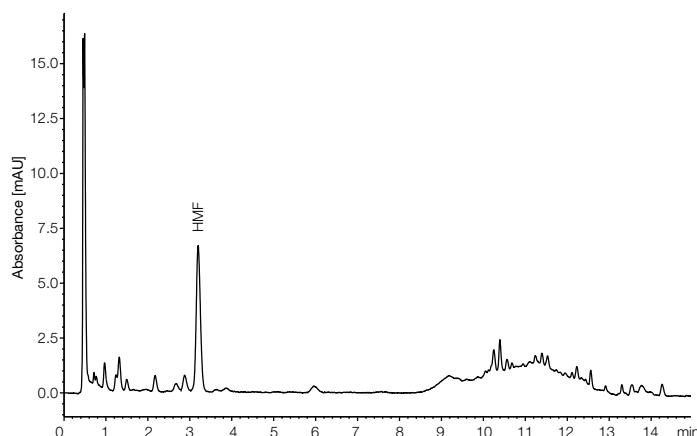


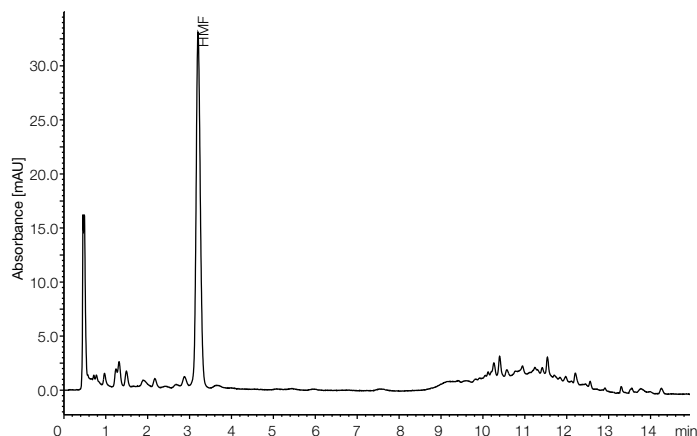
Figure 2: Chromatograms of honey samples spiked with HMF
A: 0 mg/kg, B: 2.5 mg/kg.

Sunflower



B:

Wildflower



Calibration curve

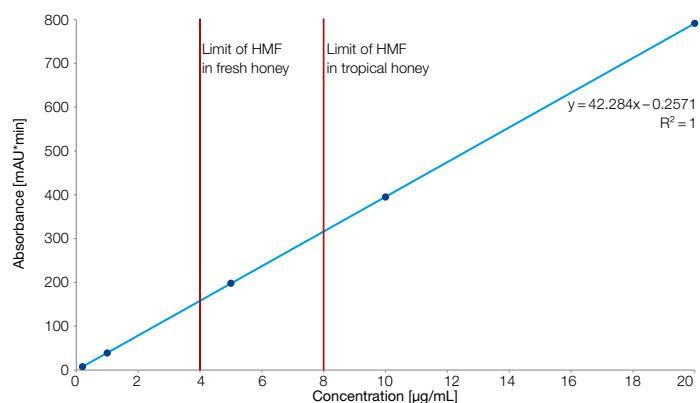


Figure 3: Calibration curve in concentration range between 0.2 µg/mL and 20 µg/mL with an excellent coefficient of determination ($R^2 = 1.0$).

Recovery rates

Sample	Recovery rate [%] (n = 3)
Wildflower honey	92.16 ± 0.80
Sunflower honey	92.96 ± 0.66

Table 1: Recovery rates for honey samples.

Conclusion

The results show that the determination of HMF from honey could be carried out successfully with RP-HPLC-UV with recovery rates for HMF of more than 92 % for both tested honey samples. The identification and quantification of HMF in the clarified sample extracts were carried out by UV detection on an EC 100/3 mm, 3.0 µm NUCLEODUR® C₁₈ Gravity column. The chromatographic results are presented in figure 2. The chromatograms show the results of clarified honey extracts spiked with 0.0 and 2.5 µg/kg HMF. With regard to the limits for HMF in honey, a calibration curve was performed in the concentration range between 0.2 µg/mL to 20 µg/mL. The calibration curve shows an excellent coefficient of determination ($R^2 = 1.0$).

In summary the presented application describes a quick and convenient method for the determination of HMF from honey.

Hydroxymethylfurfural in honey

References

- [1] 5-Hydroxymethyl-2-Furfural (HMF) – Heat-Induced Formation, Occurrence in Food and Biotransformation – a Review, Stanislaw Kowalski et al., Pol. J. Food Nutr. Sci., 2013, Vol. 63, No. 4, pp. 207-225
- [2] Honigverordnung (HonigV).
- [3] Analysis of honey - Determination of the content of hydroxymethylfurfural - Part 3: High performance liquid chromatographic method.
- [4] Untersuchung von Honig, Bestimmung des Gehaltes an Hydroxymethylfurfural, Hochleistungsflüssigchromatographisches Verfahren L 40.00 10/3.

Additional information

The following applications regarding “Determination of hydroxymethylfurfural in honey according to DIN 10751 (part 3, february 2002)” and further applications can be found on our online application database at www.mn-net.com/apps

HPLC: MN Appl. No. 128690

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760084.30, EC 100/3 NUCLEODUR® C18 Gravity, 3 µm

REF 729222, CHROMAFIL® PET-20/13

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

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