



Determination of 5-(Hydroxymethyl)-2-furaldehyd (HMF) in different honeys

Introduction

Hydroxymethylfurfural (HMF) is an organic compound which derives from dehydration of fructose, e.g. when honey is heated. Honey can be heated, for easier filling. However, in order not to damage components such as enzymes, it is not advisable to heat it up to more than 40°C. In freshly centrifuged honey, HMF is not detectable, or only in very small amounts. The HMF content increases during storage at 2–3 mg/kg per year, depending on the pH and storage temperature. With a storage temperature of 21°C, the HMF content can rise already up to 20 mg/kg in one year. The European Union calls for an HMF content of not more than 40 mg/kg for honey produced in Europe. National associations of beekeepers, such as in Belgium and Italy, are even stricter. Even the German Beekeepers Association (DIB) chose the label "Genuine German honey" for an HMF content of not more than 15 mg/kg. High performance thin layer chromatography (HPTLC) is a straightforward and economic method to analyze and quantify HMF in a complex sample matrix like honey with a minimum of sample preparation. The samples only have to be diluted in water and can subsequently be applied onto the TLC plate.

Results and Discussion

HMF can be detected under UV light at an hRf value of 82. Quantification was done using peak height after scanning the plate at 290 nm with a TLC Scanner. Figure 1 shows the overall scan after scanning the developed plate. Quantification was done using peak height (calibration curve shown in Fig. 2). Thereby, values between 2.7 mg/kg and 18.0 mg/kg were determined (Tbl. 1). Therefore one of the samples has an HMF value above the limit of the DIB.

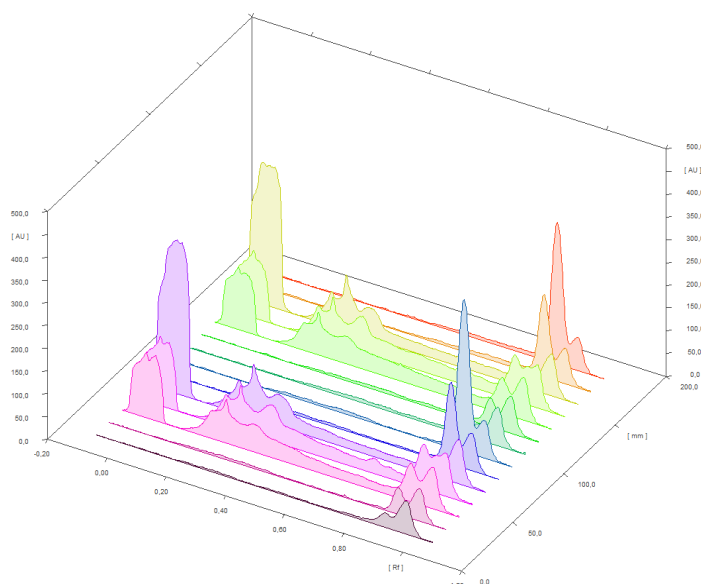


Fig. 1: Densitogram of the developed plate at 290 nm. HMF detected at Rf of 0.82 = hRf of 82.

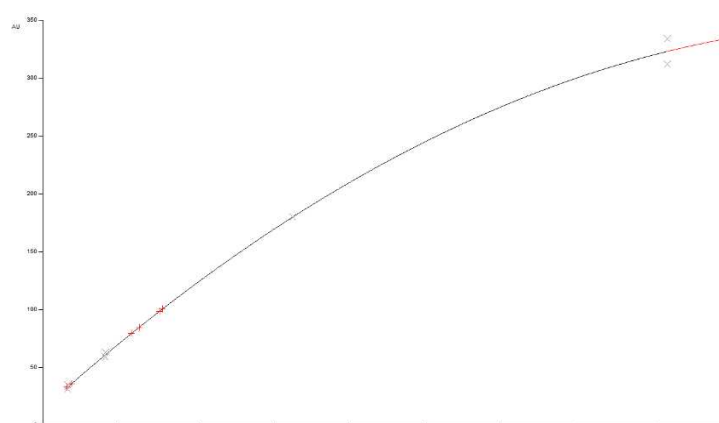


Fig. 2: Calibration curve of HMF standard (grey) and samples (red).

Conclusion

HPTLC is a straightforward method for analyzing samples with complex sample matrices as in honey. Samples only have to be diluted in water and can be applied directly to the TLC plate by an automatic sample applicator. Quantification can be done with a TLC scanner.

Tbl. 1: Determined HMF values:

Honey No 37	13.7 mg/kg
Honey No 38	18.0 mg/kg
Honey No B5	2.7 mg/kg

Chromatographic data

Track	Compound	Conc. [µg/mL]	Application volume [µL]	hRf
1, 8	HMF standard 1	1.0	6.0	82
2, 9	HMF standard 2	2.5	6.0	82
6, 13	HMF standard 3	10.0	6.0	82
7, 14	HMF standard 4	25.0	6.0	82
3, 10	Honey No. 37	250	6.0	82
4, 11	Honey No. 38	250	6.0	82
5, 12	Honey No. B5	250	6.0	82

Application data

Chromatography	Plate	HPTLC Silica gel 60 F254 MS-grade
	Sample preparation	1 g honey stirred in 10 mL Water, filtration
	Sample application	ATS 4 sample applicator (Camag), 6 mm bandwise
	Application volume	6 µL
	Mobile phase	1. Ethanol / Methanol 9/1 (v/v), 2. Ethyl acetate
	Migration distance	2 cm / 5 cm
	Migration time	1.8 min / 2.8 min
Detection	Documentation equipment	-
	Wavelength	scan under UV-light at 290 nm with TLC scanner (Camag)
	Staining	none

Ordering information:

HPTLC Silica gel 60 F ₂₅₄ MS-grade, 20x10 cm	1.00934
Ethanol gradient grade for liquid chromatography LiChrosolv®	1.11727
Ethyl acetate gradient grade for liquid chromatography LiChrosolv®	1.00868
Methanol hypergrade for LC-MS LiChrosolv®	1.06035
Millex®-FH filter, 0.45 µm hydrophobic PTFE, 25 mm, non-sterile	SLFH025NS