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In this issue:
Cleaning validation of industrial plants, sample preparation by planar chromatography, testing of honey for freshness… and more

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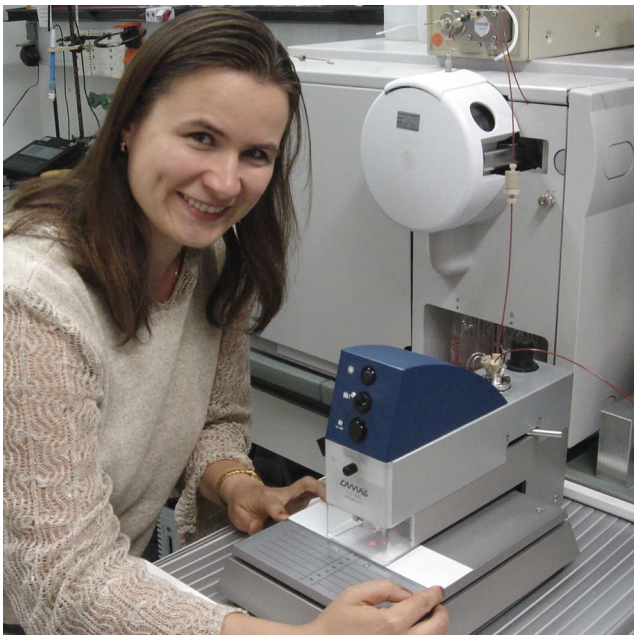
107

Fast quantification of 5-hydroxymethylfurfural in honey



18

Dr. Elena Chernetsova

In the working group of Prof. Dr. Gertrud Morlock, Institute of Food Chemistry, University of Hohenheim in Stuttgart, Dr. Elena Chemestova, guest researcher from Russia, employs planar chromatography and mass spectroscopy in her research work.

Introduction

An analytical method for quality control is expected to provide high throughput and reliability while being cost-effective. 5-Hydroxymethylfurfural (HMF) is formed by decomposition of fructose of glucose by extended storing or by heat exposure of honey. Thus the HMF content in honey is an indicator of its freshness Quantification HMF in honey is usually performed by HPLC or by spectrophotometric methods according to White or Winkler [1–4]. However, both have shortcomings. The Winkler method is comparatively imprecise, the White method uses carcinogenic reagents and is not very reliable. Thus, HPLC is usually employed, although it is fairly demanding. Samples are dissolved in water, treated with Carrez reagent to suppress decomposition of HMF, and filtrated. Then they are chromatographed one by one which takes 10–15 minutes per sample.

Planar chromatography has proven to be an efficient, fast and cost-effective alternative [5]. After minimal sample preparation, 24 samples are chromatographed side by side under identical condition, within 5 minutes and at low solvent consumption. If chromatographed from both sides in the Horizontal Developing Chamber, even 48 samples can be separated simultaneously. Reliability of the new method has been verified by TLC/MS online coupling and also by selective derivatization.

Chromatogram layer

HPTLC plates silica gel 60 or silica gel 60 F_{254}, prewashed with methanol – water 6:1 and dried 20 min at 110 °C.

Standard solutions

Aqueous solutions of HMF of 0.1, 1.0, 2.5, 5 and 10 µg/mL

Sample preparation

Honey samples are homogenized and approx. 1 g is weighted in a measuring flask and water is added at 10 mL.

Sample application

Bandwise with TLC Sampler 4 (ATS 4), track distance 7.5 mm, distance from lower edge 8 mm, application volume 1–12 µL, 24 tracks

Chromatography

Automatic Dveloping chamber (ADC2) with 10 mL ethyl acetate, migration distance 50 mm, drying time 5 min

Densitometry

TLC Scanner 3 with winCATS software, spectra recording from 200–800 nm, quantification at 290 nm, slit dimension 5 x 0.45 mm, scanning speed 20 mm/s and; depending on the working range, evaluation by polynomial or Michaelis Menten 2 regression

HPTLC-MS (optional)

The positions of the HMF zones were marked with a soft pencil. Elution with the TLC-MS Interface with circular elution head with methanol 0.2 mL/min with an inline filter (0.5 µm frit Upchurch) fitted in the outlet capillary. Mass spectra were recorded using an electrospray ionization single quadrupole mass spectrometer (ESI-MS) and the LC/MSD Chemstation (Agilent).

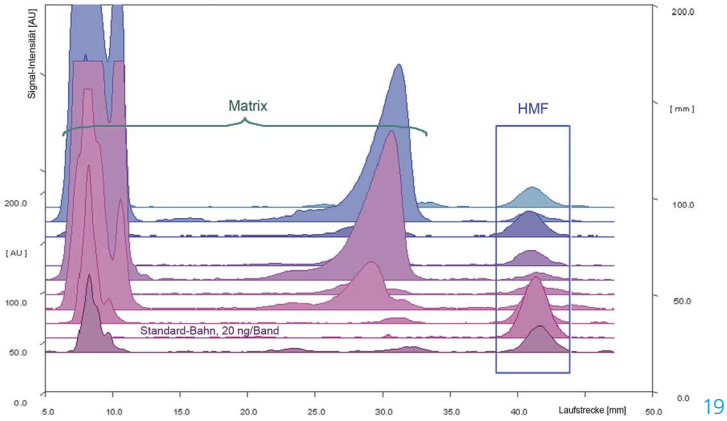
Post-chromatographic derivatization (optional)

The HPTLC plate was immersed with the TLC Immersion Device (speed 5.0 mm/s, immersion time 0 s) in *p*-aminobenzoic acid reagent (1 g dissolved in 36 mL acetic acid, then added 40 mL water, 2 mL phosphoric acid 86 % and 120 mL acetone). The plate was heated at 110 °C for 5–10 min with TLC Plate Heater.

Results and discussion

In the densitogram the HMF zones (hR_f 80) were clearly separated from the various matrix compounds of the honey samples. The identity of HMF was confirmed by UV spectra. The optimal wavelength of 290 nm for quantification was established by spectra recording.

The detection limit (LOD, S/N of 3, peak height) in the honey samples was comparable with that without matrix and corresponded to 0.75 mg/kg when 12 µL aqueous solution was applied. The limit of quantification (LOQ, S/N of 10, peak height) was 2.4 mg/kg. This proves that the method complies with the most stringent requirements worldwide, which are 15 mg/kg of HMF in honey. LOD and LOQ could be even lowered by increasing the sample volume applied.



19

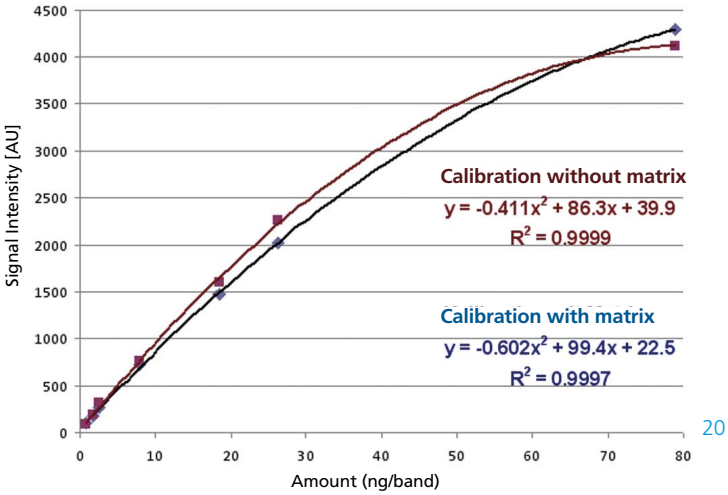
Densitogram of honey samples and HMF standard (track 2) absorbance at 290 nm

The calibration function was polynomial in a working range of 1:100, whilst Michaelis Menten 2 was suitable for higher HMF concentrations

Regression	Calibration range	Correlation coefficient r	Relative standard deviation sdv
Polynomial	1:100 (0.8–80 ng/band)	≥ 0.9998 (A) ≥ 0.9999 (H)	≤ 2.5 % (A) ≤ 1.4 % (H)
Michelis Menten 2	1:1000 (11–1100 ng/band)	n.d. n.d.	≤ 1.5 % (A) ≤ 2.3 % (H)

A peak area H peak height n.d. not defined

As matrix components obviously cause no interference, calibration is usually performed by external standards.



20

Calibration function of HMF with and without honey matrix

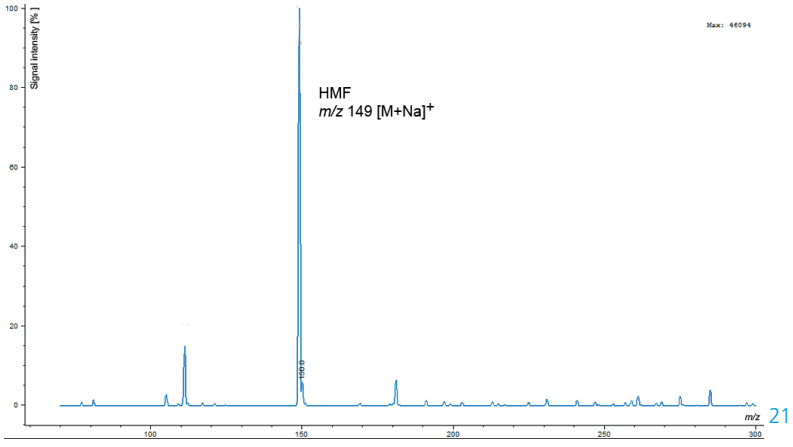
For 10 honey samples received from the Apicultural State Institute (Stuttgart) and from the Institute of Apiculture (Celle), the HMF results obtained with the Winkler method were compared with those obtained with HPLC-UV

and with the new HPTLC-UV methods. It is apparent that the differences between the two orthogonal chromatographic methods are minor (3.3 % 0.9 mg/kg), confirming the validity of the new method.

Sample #	Winkler method	HPLC-UV		HPTLC-UV		
	HMF in honey, mg/kg	HMF in honey, mg/kg	difference to Winkler method, %	HMF in honey, mg/kg	difference to Winkler method, %	difference to HPLC, % (mg/kg)
1	95.3	–	–	75.2	22	–
2	41.8	–	–	30.8	30	–
3	46.1	38.5	16	39.3	16	2.1 % (0.8)
5	17.6	13.5	23	13.7	20	1.4 % (0.2)
7	21.6	18.1	16	18.8	13	3.9 % (0.7)
8	40.2	30.4	24	28.7	26	5.6 % (1.7)
10	23.9	–	–	25.1	5	–
mean value			**20**		**19**	**3.3 % (0.9)**

The repeatability in matrix (*%RSD*, *n* = 6, peak height) was 2.9 % for a 10 ng HMF band and 06 % for a 100 ng band. The mean reproducibility (*%RSD*, *n* = 2, peak height) of the whole procedure for 4 honey samples was 3.0 % (i.e. between 1.9 and 4.4 %).

HPTLC-MS online coupling proved suitable as complementary confirmation of the HMF results. HMF zones identified by UV scanning were eluted and subjected to ESI-MS full-scan modus. The mean deviation of the HMF results between HPTLC-UV and HPTLC-MA was found at 11 % (5.1 mg/kg).



Full-scan MS of an HMF zone of a 80 ng/band honey sample

Derivatization of the HMF zones to a blue fluorescing derivative followed by fluorescence scanning at 366/>400 nm served as additional verification.

Conclusion

The verification of the results of HMF quantification in honey obtained by the new HPTLC method was successfully demonstrated by comparison with the established methods. High sample throughput and cost-efficiency combined with reliability made it apparently superior for quality control analysis.

Further information is available from the authors.

[1] S. Bogdanov *et al.* Apidologie 35 (2004) S4

[2] E. Chernetsova, I.Revelsky, G. Morlock Anal Bioanal Chem, 401 (2011) 325-332

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