Fast, Simple, and Affordable: A Better Way to Analyse HMF in Honey

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Overview & Importance of Testing for HMF

Hydroxymethylfurfural (HMF) is a recognised indicator of honey freshness and quality forming when honey is overheated or stored for extended periods. Elevated levels can signal heat damage, poor storage or adulteration making HMF monitoring essential for both quality assurance and regulatory compliance. Under Directive (EC) 110/2001, honey placed on the European market must meet strict compositional standards and contain no additives, with HMF limits set at 40 mg/kg for most honey (except bakers honey) and 80 mg/kg with an origin from tropical climate region or blend containing these honeys.

Traditional testing methods such as HPLC are accurate but costly, time-consuming, and require specialist expertise. This new test kit, based on AOAC Official Method 980.23, provides a rapid, affordable and easy-to-use alternative, delivering high precision, excellent sensitivity, and strong agreement with reference laboratory values. By enabling fast, reliable in-house testing, producers can verify compliance, detect quality issues before distribution and avoid the delays and expenses of outsourcing to third-party laboratories. Ultimately protecting product integrity, reputation, and market access.

Sample Preparation Summary:

Step 1 - Extraction of HMF from Honey Sample:

Honey samples are prepared by diluting 5 g of honey in 25 mL deionised water, vortexing to dissolve followed by the addition of 0.5 mL each of reagents R1 and R2. The sample mixture is topped up with deionised water to 50 mL, inverted to mix, and then filtered through folded Whatman paper. An initial 10 mL portion is discarded to waste, and the remaining 40 mL filtrate was collected for analysis on the PRISM analyser.

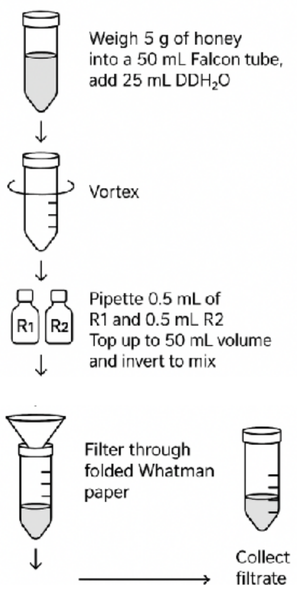

Figure 1: HMF Extraction Method

Step 2 - Sample Dilution for PRISM Analyser:

- Pipette 5 ml of filtrated honey into each of two 15 ml tubes.
- In Sample A, add 5 ml of distilled water.
- In Reference B, add 5 ml of 0.2% sodium bisulfite solution.
- Vortex both tubes for 30 seconds.

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Step 3 - Running the HMF Test on the PRISM Analyser:

Step	Absorbance (nm)
1. Power On	Switch on PRISM analyser. Warm up 20 min for internal calibration.
2. Select Test	Press Quantitation -> menu button -> Open. Select HMF Test -> Open. Press Measure to load program. (If previous program was run: press Return, then Measure.)
3. Blank Measurement	- Pipette 1 ml Reference B into quartz cuvette (no bubbles). - Place in Position 1 with opaque sides forward, clear sides parallel to lamp. - Push pull rod fully in. - Close lid -> press Zero (should read 0.0000 mg/kg).
4. Sample Measurement	- Pipette 1 ml Sample A into second quartz cuvette (no bubbles). - Place in Position 2, align with beam, push pull rod in. - Close lid -> press measurement button
5. Results	Reading appears on screen (mg/kg). Optional: connect BIOXBFD49 printer to print results.

Figure 2: PRISM Analyser

Results

Intra-Precision

Intra-assay precision was assessed using two FAPAS honey reference samples containing low and high concentrations of HMF, along with a negative control sample. Each sample was tested twenty times to evaluate the precision of the HMF assay. The coefficient of variation (CV) for all test lines was found to be below 10%.

	Level 1 FAPAS T2850QC	Level 2 FAPAS T2851QC	Level 3 Honey Sample
Mean (mg/kg)	27.23	96.44	2.0
S.D	0.556	0.477	0.104
C.V (%)	2.04	0.5	5.1
N	20	20	20

Table 1: PRECISION: Intra assay precision.

Inter-Precision

Inter-assay precision was assessed using two FAPAS honey reference samples containing low and high concentrations of HMF. Each sample was tested ten times to evaluate the precision of the HMF assay. The coefficient of variation (CV) for all test lines was found to be below 10%.

	Level 1	Level 2
Mean (mg/kg)	26.47	95.75
S.D	0.058	0.131
C.V (%)	0.22	0.14
N	10	10

Table 2: PRECISION: Inter assay precision

Recovery

To evaluate assay recovery across the full measuring range, five different honey samples were each spiked at six concentration levels. The assay demonstrated excellent recovery and linearity, with values ranging from 98% to 106%.

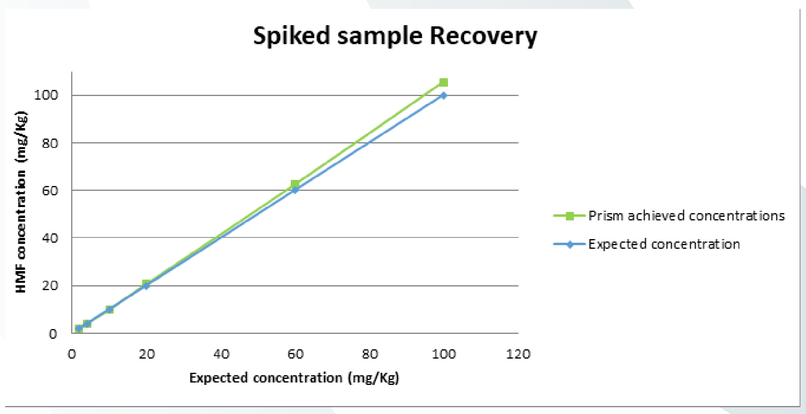

Figure 3: Spiked Recovery of HMF

Correlation with Confirmatory Methods of Analysis

To evaluate accuracy and establish correlation with confirmatory methods, ten honey samples spanning a range of HMF concentrations were analysed using the HMF assay. Results were compared directly with those obtained from the reference confirmatory method, demonstrating strong agreement across the full concentration range.

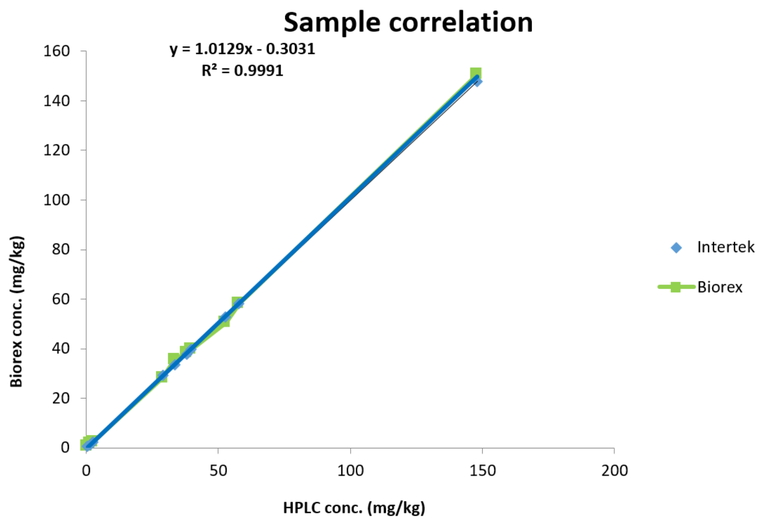

Figure 4: Correlation with Confirmatory Analysis

Limit of Detection

The Limit of Detection (LOD) was determined following standard validation procedures. Twenty independent honey samples were obtained from various supermarkets and local beekeepers and were confirmed to be freshly harvested with low HMF levels. These samples were analysed using the assay, and the LOD was calculated as the mean response of the negative samples plus three times the standard deviation (Mean + 3SD). The resulting LOD was 2 mg/kg, well below the lowest regulatory tolerance limits.

Replicate	Concentration mg/kg
1	2.12
2	2.1
3	1.96
4	1.98
5	2.3
6	2.21
7	1.99
8	2.15
9	2.22
10	1.94
11	2.09
12	1.94
13	2.05
14	2.06
15	2.05
16	1.96
17	1.95
18	2.01
19	1.98
20	1.99
MEAN	2.05
SD	0.104
%CV	5.06%
Mean +3SDs	2.4
Target	<10%
Min Acc	<20%
PASS or FAIL	PASS

Table 3: Limit of Detection

Conclusion

This developed HMF test kit offers precise (<2% variation), highly sensitive (2 mg/kg LOD), and accurate (98%–106% recovery) results using a simple process that can be performed on-site in minutes. By eliminating the need to send samples to third-party laboratories, producers can avoid high testing costs and long wait times for results. This test allows beekeepers, cooperatives, and honey processors to identify quality issues before shipping, reduce the risk of rejected consignments, and make rapid decisions to protect both product integrity and market reputation. Fast, accurate, and affordable, this kit puts quality control directly in the hands of those who need it most, supporting regulatory compliance, profitability, and customer trust.

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