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## HPLC Analysis of Caramels in Alcoholic Beverages

### Scope and Application

This method has been developed for determining the concentrations of 5-Hydroxymethyl-2-furaldehyde (5-HMF), and 2-Furaldehyde (furfural, FF) in blended and straight whiskeys, grape brandies, and rums.

Caramels are typically added to alcoholic beverages as coloring agents.

#### Regulatory Tolerances:

Caramels include Furfural and 5-Hydroxymethyl-2-Furaldehyde (5-HMF). Both compounds are found in charred oak barrels and are imparted into the distilled spirits when they are stored in them. Distilled spirits such as straight and bourbon whiskeys contain these natural caramels<sup>1</sup>.

Both 5-HMF and furfural are naturally presented in straight whiskeys as the result of storage in freshly charred oak barrels. However, synthetically produced caramel, which contains only 5-HMF is added to blended whiskeys, brandies, and rums as coloring agent.

The synthetic caramels are approved as GRAS (Generally Recognized As Safe) when used in accordance with good manufacturing practice<sup>2</sup>. These compounds are determined in straight and blended whiskeys for the purpose of distinguishing the whiskey types<sup>3</sup>.

### Levels and Limitations

Analyte	Detection Limit (mg/L)	Quantitation Limit (mg/L)	Linear Range (mg/L)	Interferences
5-HMF	0.1	0.3	1.0 – 40.0	None
Furfural	0.1	0.3	1.0 – 40.0	None

The samples are analyzed directly. However, dilution may be necessary if the analyte concentration exceeds 40.0 mg/L.

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### *Supplemental Documents*

**BAL:WG:300- Setting up the HPLC for SSD:TM:310**

**BAL:WG:301- Sample Sequence for SSD:TM:310 HPLC Analysis of 5-HMF and Furfural in Distilled Spirits**

**BAL:WG:302- Data Analysis for SSD:TM:310 HPLC Analysis of 5-HMF and Furfural in Alcohol Beverages**

### Equipment

#### Instrumentation:

Liquid chromatography system: Agilent 1200 HPLC with a Photodiode Array (PDA) Detector or equivalent system

Column: Agilent C 18, 5  $\mu$ m particle size, **250** mm x 4.60 mm id, or equivalent

Guard Column: Agilent C 18, 5  $\mu$ m particle size, 12.5 mm x 4.60 mm id, or equivalent

#### Glassware and Supplies:

Pipettors: 100  $\mu$ L, 500  $\mu$ L, 1000  $\mu$ L, 5000  $\mu$ L

Class A pipets and volumetric flasks

Disposable syringe

Syringe filter (0.45  $\mu$ m), Teflon or Nylon

LC autosampler vials (2 mL)

### Reagents, Standards, and HPLC solvents

#### Reagents

1. Deionized Water: 18 mega $\Omega$  or better
2. Methanol (CAS# 67-56-1; HPLC grade)
3. Ethanol (200 proof)
4. 5-Hydroxymethyl-2-furaldehyde (5-HMF); analytical grade
5. 2-Furaldehyde (Furfural, FF); analytical grade

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## Standards

### 1) Stock Standards:

- a. 1000 mg/L of 5-HMF stock standard: Weigh out 0.1000 ( $\pm 0.0005$ ) g of 5-HMF [Note: account for purity if the compound is not 100%], add to a 100 mL volumetric flask and dissolve it in 40% ethanol solution, and make up the volume to 100 mL with 40% ethanol solution.

Store the standard in the refrigerator for up to 12 months.

- b. 1000 mg/L of furfural stock standard: Weigh out 0.1000 ( $\pm 0.0005$ ) g of furfural (2-Furaldehyde) [Note: account for purity if the compound is not 100%], add to a 100 mL volumetric flask and dissolve it in 40% ethanol solution, and make up the volume to 100 mL with 40% ethanol solution.

Store the standard in the refrigerator for up to 12 months.

- ### 2) Working Standards:
- Prepare the working standards as outlined below. Store the working standards in the refrigerator for up to 12 months.

**Level 1** (1.0 mg/L 5-HMF and Furfural): Pipet 100  $\mu$ L of each of the two stock solutions into a 100 mL volumetric flask and make up the volume with 40% ethanol.

**Level 2** (5.0 mg/L 5-HMF and Furfural): Pipet 500  $\mu$ L of each of the two stock solutions into a 100 mL volumetric flask and make up the volume with 40% ethanol.

**Level 3** (10 mg/L 5-HMF and Furfural): Pipet 1000  $\mu$ L of each of the two stock solutions into a 100 mL volumetric flask and make up the volume with 40% ethanol.

**Level 4** (20 mg/L 5-HMF and Furfural): Pipet 2.0 mL of each of the two stock solutions into a 100 mL volumetric flask and make up the volume with 40% ethanol.

**Level 5** (40 mg/L 5-HMF and Furfural): Pipet 4.0 mL of each of the two stock solutions into a 100 mL volumetric flask and make up the volume with 40% ethanol.

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**Blank** (40% ethanol used for the preparation of standards)

**3) HPLC Solvents:** Water and methanol

## Procedures

- 1. Sample Preparation:** Samples containing alcohol above 40% are diluted with water to reduce the alcohol content to  $\leq 40\%$ . Filter all samples using a 0.45  $\mu\text{m}$  Teflon or Nylon syringe filter into a HPLC autosampler vial.

For samples that show concentrations higher than the highest standard when analyzed without dilution, appropriate dilution is necessary to bring down the concentration of the analytes below that of the highest standard.

- 2. LC/DAD Operating Procedures:**

Analyze the standards and samples in a LC/DAD with the following parameters.

- Column: Agilent C18 column, **250** mm x 4.60 mm id, 5  $\mu\text{m}$  (or equivalent)
- Organic Phase: Methanol
- Aqueous Phase: De-ionized water
- Detector: Measure the UV absorbance at wavelength 275 nm
- Column Temperature: Ambient
- Flow: 1.0 mL/min
- Injection volume: 5  $\mu\text{L}$
- Mobile phase gradient: Follow the table given below

**Table 1. Mobile Phase Gradient Table**

Time, min	Methanol %	Water %
0	5.0	95.0
1	10.0	90.0
2	20.0	80.0
13	25.0	75.0
15	35.0	65.0
16	5.0	95.0
21	5.0	95.0

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## Quality Control

1. The order of the sequence **is as per BAL:WG:301**.
2. The Laboratory Control Sample (LCS) is prepared by spiking a whiskey (straight or blended) at **approximately 20 mg/L of 5-HMF and approximately 20 mg/L of furfural**. **Record the exact spiking level on the bottle in which the LCS is stored**. Store the LCS in the refrigerator. The non-spiked portion of the same whiskey is also stored in the refrigerator. The LCS is treated as a sample and undergoes the sample preparation process as the other samples. The **shelf life** of the LCS is six months.
3. The spiked LCS should be run in duplicate, as per laboratory policy. The non-spiked LCS is run only once for determining spike recoveries.
4. The linearity of the calibration curves should be 0.99 or better.
5. If the standard curve or the control samples are not within specifications, then do the traditional LC troubleshooting and rerun the sequence.
6. Re-run the standard check (10 ppm) after every 10 samples in the sequence.
7. The tolerance for LCS and standard check is  $\pm 30\%$  of the expected value.

## Sources of Uncertainty

1. Pipetting errors
2. Column degradation
3. Any contamination during preparation

## Calculations

The elution order of the compounds in the standards is as follows: 5-HMF and furfural.

The calibration curves are determined from the peak areas of the 5 standards at 275 nm.

Dilution factor must be taken into account when calculating the final values.

$$\text{Recovery (\%)} = \frac{\text{Spiked LCS value (ppm)} - \text{Unspiked LCS value (ppm)} \times 100}{\text{Added amount (ppm)}}$$

**Acceptable recovery: 70-130%**

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## Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
5-HMF	All whiskeys, rums, brandies	mg/L (ppm)	One decimal	XX.X
Furfural	All whiskeys, rums, brandies	mg/L (ppm)	One decimal	XX.X

## Safety Notes

HPLC waste solutions and the vials must be disposed of in the appropriately labeled containers.

## References

1. Code of Federal Regulations: 27 CFR 5.21, 5.23
2. Code of Federal Regulations : 21 CFR 73.85
3. Jaganathan, J & Dugar, Sumer M. J. Assoc. Off. Anal. Chem. 82, 997 – 1001 (1999).

## Required Training and Demonstration of Competence

1. Receive in house HPLC training
2. Initial certification is achieved by running 7 LCS replicates with results of precision and accuracy in agreement with the results of the validation package
3. As determined by the Quality Manager, the chemist is retested for competency periodically.

## Revision History

Rev. 1 – Initial revision

Rev. 2- Corrected the column dimensions. Provided clarification of spiking levels for LCS; added "Supplemental Documents" section; removed sequence and referred to BAL:WG:301