Ethanol Determination by Headspace GC-MS

Scope and Application

This method will be used to quantify ethanol in beverage and nonbeverage samples using headspace gas chromatography with mass spectrometry. Samples for which this method is applicable are not distilled (as in SSD:TM:102) and are not amenable to direct injection (as in SSD:TM:217). These samples may include cakes, ice creams, pastes, syrups, powders, and other non-liquid and liquid products with high viscosity, density, and/or dissolved/suspended solids as well as low-alcohol beverages such as kombucha and non-alcoholic or alcohol-free beer.

Regulatory Tolerances:

Per 27 CFR 16.10 an alcoholic beverage is defined as a liquid which contains not less than one-half of one percent of alcohol by volume (0.5% v/v) and is intended for human consumption.

Pursuant to 27 CFR 17.133 and 17.134, the alcohol content of a product should be determined when evaluating whether the product is unfit for beverage purposes within the meaning of 26 U.S.C 5111.

Beer: 27CFR 7.65(c): Except as provided by paragraph (d) of this section, a tolerance of 0.3 percentage points will be permitted, either above or below the stated alcohol content, for malt beverages containing 0.5 percent or more alcohol by volume. However, any malt beverage that is labeled as containing 0.5 percent or more alcohol by volume may not contain less than 0.5 percent alcohol by volume, regardless of any tolerance.

27CFR7.65(d): The terms “low alcohol” or “reduced alcohol” may be used only on labels of malt beverages containing less than 2.5 percent alcohol by volume. The actual alcohol content may not equal or exceed 2.5 alcohol by volume, regardless of any tolerance permitted by paragraph (c) of this section.

27CFR7.65(e): The term “non-alcoholic” may be used on labels of malt beverages only if the statement “contains less than 0.5 percent (or .5%) alcohol by volume” appears immediately adjacent to it, in readily legible printing, and on a completely contrasting background. No tolerances are permitted for malt beverages labeled as “non-alcoholic” and containing less than 0.5 percent alcohol by volume.)).

27CFR7.65(f): The term “alcohol free” may be used only on malt beverages containing no alcohol. No tolerances are permitted for “alcohol free” malt beverages.

Levels and Limitations

This method may involve dilution of a product sample to enable mechanical homogenization and/or to decrease the alcohol content. The target range for as-analyzed samples is 0.1% v/v to 2.0% v/v ethanol. Therefore, product samples containing more than 2.0% v/v must be diluted and those containing 0.1% v/v to 2.0% v/v ethanol may include an appropriate dilution factor during sample preparation. The lower limit in % wt/wt for products not amenable to pipetting will depend on the density of the product as well as the dilution factor.

The use of a stable isotope internal standard minimizes the impact of matrix effects. However, mechanically homogenized samples which have a phase-separated lipid layer should be analyzed using SSD:TM:102 rather than this method.

Analyte	Detection Limit	Quantitation Limit	Linear Range	Interferences
Ethanol	0.003% v/v	0.009% v/v	0.1 – 2.0% v/v	none

Supplemental Documents

1. SSD:TM:102 Ethanol Determination by Specific Gravity and Specific Gravity Determination in Beverages
2. SSD:TM:217 GC-FID for the Analysis of Ethanol and Propylene Glycol in Liquid Nonbeverage Products
3. BAL:WG:103b Degassing of Beers
4. NPL:Form:200 GC-MSD Daily Log
5. NPL:Form:201 GC-MSD System Performance
6. SSD:Form:218 – SSD:TM:218 Standards Preparation Worksheet
7. NLC:Form:218 – SSD:TM:218 Sample Preparation Worksheet (spreadsheet)

Equipment

Specific vendors and model numbers are listed for convenience. Equivalent products may be used.

Instrumentation and Run Conditions:

GC:	Agilent 7890a GC with 5975 MSD and 7697A headspace sampler
Column:	J&W DB-624, 30 m × 0.25 mm × 1.4 µm (P/N 122-1334)
Carrier Gas:	Helium, 1 mL/min constant flow
Temperature Program:	45 °C initial, hold 3 min, ramp at 25 °C/min to 240 °C, hold 3 min
GC Injector:	200 °C; 200:1 split
MS Inlet:	240 °C
MS Scan:	29-250 m/z at 6.1 scans/s
HS Injection:	50 µL loop; 0.5 s
HS Temperatures:	Vial oven 70 °C; Sample loop 85 °C; Transfer line 95 °C
HS Equilibration:	10 min; shaker setting 5 (71 min^{-1}); vial pressure 20 psi
Software:	Agilent MassHunter B.09.00

Ion Type	ethanol		ethanol-d6 (ISTD)	
	Mass	Abundance	Mass	Abundance
Quantifier	31	100	33	100
Qualifier 1	45	75.9	49	57.3
Qualifier 2	46	32.1	51	39.0

Density Metering Apparatus (DMA)

Anton Paar DMA 5000, temperature controlled to 20.00 ± 0.01 °C and reporting density to five decimal places (±1 in the last decimal place) and ethanol % v/v according to AOAC at 60 °F. Please refer to SSD:TM:102 for information about the appropriate use of the DMA.

Analytical Balance

An analytical balance (Sartorius, 1602 MP8-1) is used in the preparation of samples for analysis of ethanol % wt/wt. Any balance used for such purpose should be accurate to ±0.1 mg.

Mechanical Homogenizer

A mechanical homogenizer (Fisher, 150) with 10 mm stainless steel generator probe is used for the preparation of samples.

Glassware and Supplies:

- Clear or amber glass bottles with conical caps, 2-4 oz. (Fisher, 02-911-353)

- Micropipettes and tips – 2-200 µL, and 50-1000 µL (Fisher, 02-717-351 and 02-717-352)
- Positive displacement pipet – 1000 µL (Fisher, 13-688-253)
- 20-mL headspace vials and caps (Agilent, 5183-4478 and 5182-0837)
- DMA tubes and caps (Fisher, 03-341-4 and 02-544-27)
- Kimwipes (Fisher, 06-666A)
- 15- and 50-mL centrifuge tubes (Fisher, 12-565-269 and 12-565-271)

Reagent and Sample Preparation and Handling

Specific vendors and product numbers are listed for convenience. Equivalent products may be used.

Reagents:

- Deionized water (DI water), 18.0 MΩ·cm or better
- Ethyl alcohol (CAS # 64-17-5, 200 proof, Pharmco-Aaper)
- Ethyl alcohol-d6 (CAS # 1516-08-1, >99.5 atom %, Sigma- Aldrich, 186414)

Preparation of Internal Standard (1% v/v ethanol-d6 in DI water):

1. Weigh an empty 2-oz bottle.
2. Pipet 500 µL of ethyl alcohol-d6 into the bottle and reweigh.
3. Add 50 g of DI water and mix well.
4. The solution may be stored at 4 °C for up to one year.

Preparation of Calibration Standards:

Use the scheme outlined below to prepare the calibration standards. The values measured during preparation of the solutions should be recorded in the worksheet listed in the Supplemental Documents section.

1. Weigh an empty 4-oz bottle.

2. Pipet the desired volume of 200 proof ethanol into the bottle and reweigh.

Solution	Target [EtOH] (% v/v)	EtOH volume (µL)	EtOH weight (g)
Cal 1	0.10	100	0.079
Cal 2	0.25	250	0.197
Cal 3	0.50	500	0.395
Cal 4	1.00	1000	0.789
Cal 5	1.50	1500	1.184
Cal 6	2.00	2000	1.579

3. Add DI water to bring the total weight to 100 g and mix well.
4. Determine the density and alcohol concentration (% v/v) of each solution using a densitometer (refer to SSD:TM:102).
5. The solutions may be stored at 4 °C for up to one year.

Preparation of Frozen LCS:

The frozen LCS is a commercial ice cream spiked with ~0.6% wt/wt ethanol.

1. Weigh a 100 g portion of ice cream into a 500-mL beaker.
2. Add 200 g of DI water.
3. Add 2 g of 200 proof ethanol.
4. Homogenize the mixture using a mechanical homogenizer.
5. Transfer 5-mL aliquots into individual 50-mL centrifuge tubes.
6. The tubes should be stored at -20 °C for up to three years.

Liquid LCS:

The liquid LCS is a canned, non-alcoholic beer (e.g. O'Doul's). Cans should be stored at 4 °C for up to three years.

Procedures

Preparation of Samples:

Follow one of the schemes outlined below to prepare samples for analysis. The calculations used for sample preparation are described in the Calculations section and a worksheet to perform those calculations is listed in the Supplemental Documents section.

Homogenized Sample Preparation

For samples that are amenable to mechanical homogenization with water.

1. Determine an appropriate dilution factor based on the expected/known alcohol content in the sample.
2. Weigh a representative portion of sample into a 50-mL centrifuge tube or beaker (depending on size).
3. Weigh an appropriate amount of water into the vessel to achieve the desired dilution.
4. Homogenize the mixture using a mechanical homogenizer.

Liquid Sample Preparation

1. If the sample is carbonated, degas as per specific lab guidelines.
2. Determine an appropriate dilution factor based on the expected/known alcohol content in the sample.

For samples which do not require dilution, use the sample as-is. Otherwise, dilute as follows.

3. Pipet an appropriate volume of sample into a 15-mL centrifuge tube.
4. Pipet an appropriate volume of DI water to achieve the desired dilution into the tube and mix well.

Preparation of Vials

It is important that all vials in an analysis sequence contain the same volume. For routine analysis, the total volume should be 1.1 mL. All solutions should be removed from the cold room/freezer approximately two hours prior to vial preparation to allow everything to reach room temperature. The calculations used for vial preparation are described in the Calculations section and a worksheet to perform those calculations is listed in the Supplemental Documents section.

Calibration Standard Vial Preparation

1. Pipet 1000 µL of standard into a 20-mL headspace vial.
2. Pipet 100 µL internal standard solution into the vial and crimp on a cap.

Frozen LCS Vial Preparation

Prepare vials in duplicate from a single tube of LCS.

1. Homogenize the contents of the centrifuge tube using the mechanical homogenizer.
2. Weigh an empty 20-mL headspace vial.
3. Use a positive displacement pipet to transfer 1000 µL of the mixture into the vial.
4. Reweigh the vial.
5. Pipet 100 µL of internal standard solution into the vial and crimp on a cap.

Liquid LCS Vial Preparation

Prepare vials in duplicate from a single can of LCS.

1. Pour an approximately 15 mL aliquot from a freshly opened can into a 50-mL centrifuge tube.
2. Degas the solution using the mechanical homogenizer.
3. Pipet 1000 µL of degassed LCS into a 20-mL headspace vial.
4. Pipet 100 µL of internal standard solution into the vial and crimp on a cap.

Homogenized Sample Vial Preparation

1. Weigh an empty 20-mL headspace vial.
2. Use a positive displacement pipet to transfer 1000 µL of homogenized mixture into the vial.
3. Reweigh the vial.
4. Pipet 100 µL of internal standard solution into the vial and crimp on a cap.

Liquid Sample Vial Preparation

1. Pipet 1000 µL of sample or diluted sample into a 20-mL headspace vial.
2. Pipet 100 µL of internal standard solution into the vial and crimp on a cap.

Quality Control

1. The coefficient of determination for the calibration standards should be ≥ 0.995. If the coefficient of determination is below 0.995, re-run using freshly prepared vials of calibration standards. If the coefficient of determination is still below 0.995, contact a co-principal analyst.
2. Whenever samples are prepared, at least one LCS should be prepared in duplicate and analyzed as part of the sequence. Choice of appropriate LCS should be based on the sample types in the sequence. If either LCS has not been analyzed in more than four weeks, that LCS should be included in the sequence regardless of sample types.
3. If a single LCS replicate falls outside the accuracy range, prepare a new duplicate set of vials for that LCS and re-run the sequence. If all LCS replicates pass then report the data; if any of the LCS replicates fail then tag the instrument out of service. Any LCS failure requires the initiation of a CAR (refer to SSD:QPD:310 for more information).

Sources of Uncertainty

1. Incomplete homogenization of sample.
2. Non-representative aliquot of sample.
3. Weighing error during sample or vial preparation.
4. Pipetting error during sample or vial preparation.

Calculations

Instrument software is used to generate a 1/x weighted linear least squares calibration curve and to calculate the concentrations (% v/v or % wt/wt) for samples based on dilution and unit conversion factors calculated during sample preparation. The following calculations are automatically performed using NLC:Form:218 once all data is entered into the correct fields.

For determining the unit conversion factor to convert measured concentration in % v/v to % wt/wt, divide the density of ethanol at 60 °F by the weight of sample added to the vial. The weight of sample in the vial is the difference between the empty vial weight and the weight of the vial after adding sample. For determining the dilution factor for homogenized samples, multiply the unit conversion factor by the total weight of the homogenized mixture and divide by the weight of sample in the mixture. For determining the dilution factor for liquid samples, divide the total volume by the volume of sample.

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
Ethanol	Non-beverage	% v/v % wt/wt	1 decimal	*X.x*
Ethanol	Beverage	% v/v	2 decimals	*X.xx*

Safety Notes

High proof ethanol is flammable and burns with an almost invisible blue flame. Use extreme caution when working with high proof ethanol.

Samples and/or in-house prepared standards waste may be disposed down the drain. Waste containing internal standard must be disposed of using the flavor waste stream. Used headspace vials must be disposed of using the GC/LC vial waste stream.

References

1. Electronic Code of Federal Regulations, Title 27. https://www.ecfr.gov (accessed 3/20/2020).

Required Training, Certification and Re-certification

1. Receive in-house HS-GC training.
2. Initial certification is achieved by running 7 replicates of each LCS with results of precision and accuracy in agreement with the results of the validation package

3. Chemists are retested for competency (e.g. every 5 years) and/or given a proficiency test.

Revision History

Rev. 1 – initial revision

Rev. 2 – Updated storage period for frozen LCS and frequency of LCS analysis.