Identity Confirmation of Ethanol in Alcohol Products by Gas Chromatography-Mass Spectrometry

Scope and Application

The qualitative method will be used to confirm the identity of ethanol in SSD alcohol enforcement samples. It will be used primarily to identify the presence of ethanol in suspected moonshines or for the presence of alcohol in samples that are represented as "nonalcoholic". The method can also be used to confirm the identity of ethanol in NPL enforcement samples such as cooking wines. The samples will be diluted in methanol then analyzed by GCMS. The identity of ethanol in the sample is performed by comparing the mass spectrum of the peak in the sample to the mass spectrum for ethanol from the NIST library. The method will be used to analyze samples for which the alcohol content is known or was previously determined using other analytical techniques such as a densitometer, GC-TCD or GC-FID.

Regulatory Tolerances:

27 CFR 16.10 defines an alcoholic beverage as any beverage in liquid form that contains not less than one-half of one percent (0.5%) of alcohol by volume and is intended for human consumption.

Levels and Limitations

The method applies to products containing about 0.4 – 100% alcohol by volume.

Supplemental Documents

NLC:WG:215 – Data Processing on Instrument NLC_2_003 for SSD:TM:215
SSD:TM:102 – Ethanol Determination by Specific Gravity and Specific Gravity Determination in Beverages
SSD:TM:103 – Specific Gravity and Density Determination in Nonbeverage Products
SSD: TM: 217 -- GC-FID for the Analysis of Ethanol and Propylene Glycol in Liquid Nonbeverage Products
SSD: TM: 218 – Ethanol Determination by Headspace GC-MS

Equipment

Instrumentation:
GC/MS: Agilent 7890B Gas Chromatograph with a 5977A Mass selective Detector or equivalent
Shaker (optional): Vortexer shaker or equivalent
Centrifuge: Eppendorf Centrifuge 5702 or equivalent

Glassware and Supplies:
Conical tubes (e.g., 15mL)
Automatic pipettors (e.g., 10 to 100µL, 1 to 10mL)
Eppendorf pipet tips or equivalent
Class A pipets (various sizes)
Volumetric flasks (various sizes)
Graduated cylinders (various sizes)
Glass or plastic bottles (optional)
2-mL GC vials with crimp seal caps

GC/MS Instrumental Procedures:

- *Column*: Stabilwax-DB 30m x 0.25mm x 0.25µm film thickness or equivalent
- *Oven:* 40ºC for 3 min; ramp @ 30ºC/min to 150ºC, hold for 1 min; ramp @ 25ºC/min, hold for 3 min. (total run time: 14.67 min)
- *Carrier:* Helium, Constant flow at 1 mL/min
- *Inlet:* 220ºC; Split 100:1
- *Injector:* 5-µL or 10-µL syringe
- *Injection volume:* 0.5-µL
- *Solvent Wash:* Methanol
- *MSD:* Scan mode
- *Solvent delay:* At least 3.00 minutes
- *Mass range:* 20 – 100 amu

Reagent, Standard and Sample Preparation

Reagents:
Ethanol, 200 Proof
Deionized (DI) water or equivalent
Methanol, HPLC Grade or equivalent

Preparation of Standards and Sample Solutions:
Note: Volumes other than indicated below for preparing the Standard Stock, Working Standard Solution and sample solutions may be used as long as the final concentrations are the approximately the same.

Prepare the following Ethanol ID Standard Stock and Working Standard Solution:

1. **Ethanol ID Standard Stock:** Prepare a 40% Ethanol ID Standard Stock in water. For example, add 40mL of ethanol (200 proof) to a 100-mL volumetric flask and dilute the flask to volume with DI water. Mix well by hand or by vortex. If desired, transfer the Ethanol ID Standard Stock to a glass or plastic bottle for storage. The Ethanol ID Standard Stock is stable for 1 year at room temperature.
2. **Working Standard Solution:** Prepare a 1:100 ethanol:methanol Working Standard Solution. For example, pipet 100µL of the 40% Ethanol ID Standard Stock into a 15-mL conical tube and add 9.9mL of methanol to the tube. Mix well by hand or by vortex. The Working Standard Solution is stable for 5 days at room temperature.
3. **Sample Solutions:** To prepare the sample solutions, dilute each sample in methanol based on the sample's known percent alcohol by volume (%ABV). For turbid samples or samples containing high amounts of undissolved solid matter (e.g., fermented mash), centrifuge the neat sample for about 5 minutes at high speed (e.g., 4400 rpm) and dilute the supernatant in methanol. The table below shows suggested dilution schemes of the sample in methanol to use based on the %ABV of the sample. Example sample preparation procedures are also provided in the text section following the table.

Sample ABV Content	Dilution in Methanol	Final Target Conc.
≥50 - 100%	1:200	≥0.25 – 0.5%
≥ 20 - <50%	1:100	≥0.2 - <0.5%
≥ 5 - <20%	1:50	≥0.1 - <0.4%
0.4% - <5	1:5	0.08 - <1.0%

   a. **1:200 Sample:Methanol Solution*:*** For samples containing ≥50 – 100 %ABV, prepare a 1:200 sample:methanol solution. For example, pipet 50µL of the sample into a 15-mL conical tube and add 9.95mL of methanol to the tube. Mix well by hand or by vortex. Transfer diluted solution to a 2-mL GC vial and crimp vial cap.
   b. **1:100 Sample:Methanol Solution*:*** For samples containing ≥20 - <50 %ABV, prepare a 1:100 sample:methanol solution. For example, pipet 100µL of the sample

into a 15-mL conical tube and add 9.9mL of methanol to the tube. Mix well by hand or by vortex. Transfer diluted solution to a 2-mL GC vial and crimp vial cap.

c. **1:50 Sample:Methanol Solution*:*** For samples containing ≥5 - <20 %ABV, prepare a 1:50 sample:methanol solution. For example, pipet 200µL of the sample into a 15-mL conical tube and add 9.8mL of methanol to the tube. Mix well by hand or by vortex. Transfer diluted solution to a 2-mL GC vial and crimp vial cap.

d. **1:5 Sample:Methanol Solution*:*** For samples containing 0.4 - <5 %ABV, prepare a 1:5 sample:methanol solution. For example, pipet 2mL of the sample into a 15-mL conical tube and add 8 mL of methanol to the tube. Mix well by hand or by vortex. Transfer diluted solution to a 2-mL GC vial and crimp vial cap.

Note: All above sample solutions are stable for 5 days at room temperature.

Procedures

1. Analyze the Working Standard Solution and sample solutions on the GCMS instrument using the previously mentioned GC/MS parameters:
2. Perform 2 injections of methanol blank and 1 injection of the Working Standard Solution at the beginning of the sequence.
3. Perform 1 injection of methanol blank after no more than 10 injections of the sample solutions and 1 injection of methanol blank at the end of the sequence.
4. Example Run Sequence:

Sample ID	Sample Name
Solvent Blank	Methanol
Solvent Blank	Methanol
WSB-17-999	Working Standard
B-2017-09999-001	Sample Solution
B-2017-09999-002	Sample Solution
B-2017-09999-003	Sample Solution
B-2017-09999-004	Sample Solution
B-2017-09999-005	Sample Solution
B-2017-09999-006	Sample Solution
B-2017-09999-007	Sample Solution
B-2017-09999-008	Sample Solution
B-2017-09999-009	Sample Solution
B-2017-09999-010	Sample Solution
Solvent Blank	Methanol

5. The ethanol peak in the standard elutes after the methanol solvent peak.

Calculations

1. Chemstation or an equivalent software package is used to determine the mass spectrum of the ethanol peak in the standard and samples. The ethanol peak elutes at about 3.3 minutes.
2. The presence of ethanol in the standard and samples is confirmed by comparing the mass spectrum (background subtracted) of the ethanol peak in the standard or sample to the mass spectrum for ethanol in the NIST library.
3. There are no concentration calculations for ethanol (qualitative method).

Quality Control

1. The mass spectral report will identify the ethanol peak in the Working Standard Solution at a probability of ≥ 80.
2. The chromatogram for at least 1 methanol blank will show the **absence** of an ethanol peak.

Reporting Results

1. For mass spectral probability results for ethanol ≥ 70, report the sample results as “Detected.”
2. For mass spectral probability results for ethanol < 70 or the absence of an ethanol peak in the chromatogram, report the sample results as “Not Detected.”

Sources of Uncertainty

1. Sample preparation and/or dilution errors
2. Impure and/or contaminated standards and/or reagents
3. Poor instrument performance (e.g., column condition, septa, syringe, inlet liner)

Safety Notes

1. All chemicals are considered hazardous. Avoid direct physical contact.
2. Consult the SDS for any chemicals used that are unfamiliar.
3. Wear a laboratory coat, safety glasses and protective gloves.
4. Methanol should be handled under a fume hood. A respirator or disposal mask may also be worn if handling methanol outside a fume hood.
5. The waste stream will consist of solutions containing ≤1.0% ethanol in 100% methanol. Dispose all standard solutions, sample solutions and sample vials into the appropriate laboratory hazardous waste containers.

References

None

Location of Validation Package

Quality System Files

Required Training and Demonstration of Competence

1. GC/MS operation training.
2. In-house training on the standard and sample preparation is required.
3. Initial certification is achieved by analyzing at least 2 unknown samples with or without the presence of ethanol.
4. Periodically, chemist are retested for competency (e.g. every 5 years) and/or may be given a proficiency test.

Revision History

Rev. 1 – Initial revision
Rev. 2 – Added supplemental documents.