Capillary GC Analysis of Fusel Oils and Other Components of Interest

Scope and Application

Methanol, acetaldehyde, ethyl acetate and fusel oils (F.O.) are natural fermentation products. Fusel Oils consist of n-propanol, iso-butanol, n-butanol, and amyl alcohol (2-methyl-1-butanol and 3-methyl-1-butanol). Absence of these compounds in products suggests either non-fermented products or the use of neutral spirits. Acetic acid is indicative of spoilage. Benzaldehyde and propylene glycol (greater than 0.01%) are indicative of flavoring/adulteration. Glycerol (1,000-2,000 ppm) is present naturally in wines.

This method may be used for the analysis of most alcohol beverages and nonbeverage alcohol (NBA) products with the following caveats:

1. NBA products which are NOT miscible with water cannot be analyzed by this method.
2. NBA products containing >10% solids must be diluted prior to analysis for nonbeverage analytes.
4. 3. Distilled spirit products containing >10% solids are diluted (or distilled) prior to analysis. Liqueur products are distilled prior to analysis.
5. Acetic acid, benzaldehyde, propylene glycol, and glycerol cannot be determined after distillation.

Regulatory Tolerances:

Methanol—0.1 % by volume max. in wine (Industry Circular IC-93-3)(CPG 7119.09 Section 510.200).

Methanol—0.35 % by volume max. in brandy (FDA Administrative Guides 7401.01 and 1701.01)(Topical Digest 1710.41-43)(CPG7119.09)

Fusel Oil—less than 20 ppm indicates neutral spirits (Commodity Classification Branch 4/4/1983)

Volatile Acidity/acetic acid (27CFR4.21) —

0.14 % by volume max. in **red wine** when starting brix ≤ 28

0.17 % by volume max. in **red wine** when starting brix is >28

0.12 % by volume max. in **white wine** when starting brix ≤ 28

0.15 % by volume max. in **white wine** when starting brix is >28

Propylene Glycol – For NBP's, ±5% of the stated value. The finished alcohol beverage may not contain more than 5% PG (21 CFR 184.1666).

Acetic Acid – For NBP's, ±5% of the stated value. The finished alcohol beverage may not contain more than 0.15% acetic acid (21 CFR 184.1005).

Levels and Limitations

Analyte	Detection Limit	Quantitation Limit	Calibration Range	Validated Linear Range	Interferences
Ethyl Acetate	1.8 mg/L (0.18 g/100L)	5.9 mg/L (0.59 g/100L)	9-900 mg/L (0.9-90.0g/100L)	5.9-20000 mg/L (0.59-2000 g/100L)	None
Methanol	0.0004 %	0.005 %	0.015-1.5 %	0.01-20%	None
n-Propanol	0.5 mg/L (0.05 g/100L)	1.6 mg/L (0.16 g/100L)	3-300 mg/L(0.3-30g/100L)	1.6-10000 mg/L (0.16-1000 g/100L)	None
iso-Butanol	0.5 mg/L (0.05 g/100L)	1.7 mg/L (0.17 g/100L)	6-600 mg/L(0.6-60g/100L)	1.7-20000 mg/L (0.17-2000 g/100L)	None
n-Butanol	0.3 mg/L (0.03 g/100L)	0.9 mg/L (0.09 g/100L)	3-300 mg/L(0.3-30g/100L)	1.25-2000 mg/L (0.125 – 200 g/100L)	None
Amyl Alcohol	0.3 mg/L (0.03 g/100L)	1.0 mg/L (0.10 g/100L)	12-1200 mg/L(1.2-120g/100L)	2-40000 mg/L (0.2-4000 g/100L)	None
Acetic Acid **	0.001 g/100mL	0.0032 g/100mL	0.01-0.20 g/100mL	0.002-0.2 g/100mL	Furfural
Benzaldehyde	0.0014 g/100mL	0.0046 g/100mL	0.01-0.20 g/100mL	0.002-0.2g/100mL	None
Propylene Glycol	0.0010% by Vol	0.0033 % by Vol	0.01-0.20 % by Vol	0.002-0.2% by Vol	None
Glycerol	0.006 % by Vol	0.019 % by Vol	0.05-1.00 % by Vol	0.01-1% by Vol	None
Acetaldehyde	4.9 ppm	16 ppm		40-2000 ppm	None

**** When distilled, Acetic Acid in the presence of ethanol may react to form ethyl acetate.**

Equipment

Instrumentation:

GC:	Hewlett Packard 6890 with 7673 Autosampler and Chemstation Software, or equivalent
Column:	DB-WAXETR, 30m x 0.53mm x 1μm film thickness
Carrier Gas:	Hydrogen, from generator, Proton Model G600 or equivalent, constant flow, 5.9 ml/min
Temperature:	40°C initial, hold 5 min, ramp at 10°/min to 215°C, hold 2.5 min.
Injector:	220°C, 5:1 split
Detector:	FID 250°C, Hydrogen flow 40 ml/min, air flow 450 ml/min
Injection Volume:	1 µL

Glassware and Supplies:

Class A pipets /Micropipetes
Class A volumetric flasks

Reagent and Sample Preparation and Handling

Reagents: (All chemicals for standards are 99.0+% pure.)

40% Ethanol/Water
Ethyl Acetate
n-Propanol
iso-Butanol
n-Butanol
2-methyl-1-butanol
(active Amyl alcohol)
Methanol
Propylene Glycol
Glycerol
Acetic Acid
Benzaldehyde
Acetaldhyde
200 Proof Ethanol

Preparation of Fusel Oil (F.O.) stock, working, and second source standards:

1. Prepare a **F.O. stock standard solution**. Weigh the following into a 100 mL volumetric flask and quantitate to volume with >95% Ethanol by volume. Stock solution is stable in the refrigerator for up to 12 months.

    a. 3.00 g ethyl acetate ± 1.0%
    b. 1.00 g n-propanol ± 1.0%
    c. 2.00 g iso-butanol ± 1.0%
    d. 1.00 g n-butanol ± 1.0%
    e. 4.00 g active amyl alcohol ± 1.0%

f. 50.00 mL methanol ± 1.0%

2. Prepare F.O. working standards by transferring stock or diluted standard as outlined below. The standards may be stored in the refrigerator for up to 3 months.

F.O. Level 4: Transfer 6 mL standard stock solution into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.

F.O. Level 3: Transfer 1 mL standard stock solution into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.

F.O. Level 2: Transfer 5 mL Level 4 into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.

F.O. Level 1: Transfer 2 mL Level 4 into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume

3. Prepare F.O. second source stock and working solution (F.O. Level 3 concentration) in the same manner as step 1 and 2.

This preparation results in F.O. standards with the following concentrations:

Analyte	Stock Std. ppm	FO4 ppm	FO3 and 2nd source ppm	FO2 ppm	FO1 ppm
Ethyl Acetate	30,000	900	150	22.5	9
n-propanol	10,000	300	50	7.5	3
Iso-butanol	20,000	600	100	15	6
n-butanol	10,000	300	50	7.5	3
Amyl alcohol	40,000	1200	200	30	12
Methanol	500,000	15000 (1.5%)	2500 (0.25%)	375 (0.037%)	150 (0.015%)

Preparation for NBA stock and working standards:

1. Prepare a **NBA standard stock solution** to proper concentration. Place the amounts below into a 1 L volumetric flask. Q.S. with 40% Ethanol by volume. Store stock solution in the refrigerator for up to 12 months.
    a. 2.07 g Propylene Glycol ± 1.0%
    b. 12.50 g Glycerol ± 1.0%
    c. 2.00 g Acetic Acid ± 1.0%
    d. 2.00 g Benzaldehyde ± 1.0%

2. Prepare the following **NBA working standards**:

    NBA Level 3: NBA Stock Standard, as is.

    NBA Level 2: Pipet 5 mL of NBA standard stock solution into a 10 mL volumetric flask. Q.S. with water. Prepare daily.

    NBA Level 1: Pipet 0.5 mL of NBA standard stock solution into a 10 mL volumetric flask. Q.S. with water. Prepare daily.

This preparation results in NBA standards of the following concentrations:

Analyte	Units	Stock/Level 3	Level 2	Level 1
propylene glycol	% by Vol	0.2	0.1	0.01
glycerol	% by Vol	1.0	0.5	0.05
acetic acid	g/100mL	0.2	0.1	0.01
benzaldehyde	g/100mL	0.2	0.1	0.01

Preparation for Acetaldehyde standard:

1. Prepare an **Acetaldehyde Standard** by pipeting 1 mL of acetaldehyde into a 1000 mL volumetric flask. Q.S. with 40% Ethanol by volume. Prepare daily.

Procedures

1. Run a blank to assure the system is clean. Acceptable if results are less than LOQ for all analytes being reported. If unacceptable, inspect liner, syringe/plunger and replace if necessary.

2. Run standards on the GC. The choice of standards depends on the sample type and is typically as follows:

For **beer and DSP**, use Fusel Oil Standards 1 - 4
For **wine**, use Fusel Oil Standards 1 – 4. NBA Level 1 – 3 is used when an acetic acid value is required.
For **nonbeverage products**, use NBA Level 1 - 3 and/or Fusel Oil Standards 1 - 4.
For **moonshine**, use Fusel Oil Standards 1 - 4 and the Acetaldehyde Standard.

3. Calibrate based on area using external standards on all levels for each component. The correlation coefficient (r^2) shall be > 0.99.

4. Run a blank after the highest standard to assure the system is free from carryover. Acceptable if results are less than the LOQ for all analytes being reported. If unacceptable, inspect liner, syringe/plunger and replace if necessary.

5. Run a second source check to verify the system, then inject samples. Bracket the samples with the second source check after every ten (10) samples and at the end of the sequence as a control to check for drift

6. If sample results are above the calibration range, dilute into range and re-inject.

7. Inject a blank after a possible extremely "dirty" sample to assure the system cleans out properly prior to next injection. Acceptable if results are less than LOQ for all analytes being reported. If unacceptable, inspect liner, syringe/plunger and replace if necessary.

Quality Control

1. The correlation coefficient of the external standards is to be greater than 0.99. If the correlation coefficient is not >0.99, change the liner and/or septa and rerun. If the correlation coefficient is still not >0.99, re-run using fresh working standards. If the correlation coefficient remains out of spec, contact the principal analyst.

2. Run an LCS check in duplicate. The accuracy and precision values are to be within the prescribed limits.

3. If the second source standard differs by >15% of the expected value, repeat the sample injections performed since the last passable control result.

4. Violative results are confirmed as follows:

   Propylene Glycol and Glycerol - confirm using LC Acids Method.
   Acetic Acid - confirm using a TTB Official method (SSD:TM:502 or SSD:TM:503).

5. For precision quality control failures, inspect the liner and replace if necessary.

6. ***If neat LCS values fall outside the acceptable accuracy range for at least two out of three results, re-run the calibration curve using a fresh aliquot of the calibration standards.***

Sources of Uncertainty

1. Weighing errors for standards
2. Preparation of working standards (e.g., Dilution, pipet, etc.)
3. Dirty injection liner
4. Problem with GC syringe (e.g., dirty syringe or bad plunger)
5. Change in analyte retention time

Calculations

GC is operated in external standard mode with calculations using peak areas.
Total Fusel Oil is the sum of n-propanol, iso-butanol, n-butanol and amyl alcohols.

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
Acetaldehyde	All	mg/L (ppm)	No decimal	XX
Acetic Acid	All	g/100mL	2 decimals	X.xx
Benzaldehyde	All	g/100mL	***1*** decimals	X.x
Ethyl Acetate	DSP	g/100L	1 decimal	X.x
Ethyl Acetate	Wine, Beer, NBA	mg/L (ppm)	No decimal	XX
Fusel Oils (Total and individ. components)	DSP	g/100L	1 decimal	X.x
Fusel Oils (Total and individ. components)	Wine, Beer, NBA	mg/L (ppm)	No decimal	XX
Glycerol	All	% by volume	***2*** decimals	X.xx
Methanol	All	% by volume	***2*** decimals	X.xx
Propylene Glycol	All	% by volume	2 decimals	X.xx

Safety Notes

Consult the MSDS for any chemicals used that are unfamiliar. All chemicals shall be considered hazardous - avoid direct physical contact.

Hydrogen is **explosive** and is used as a carrier gas. Extreme caution shall be used when working with the GC hardware.

If the GC is not equipped with a Hydrogen leak sensor to automatically shut down the GC, this method shall not be used.

References

Kelly et al.: “Gas Chromatographic Determination of Volatile Congeners in Spirit Drinks: Interlaboratory Study”, J of AOAC Int., Vol. 82, No. 6, 1999, pp 1375-1388.

Martin, G.E., Burggraff, J.M., Dyer, R.H., and Buscemi, P.C., “Gas-Liquid Chromatographic Determination of Congeners in Alcoholic Products by Gas Chromatography/Mass Spectrometry”, J of AOAC Int., Vol 64, January 1981, pp 186-190.

DiCorcia, A., Samperi, R., Sebastiani, E. and Severini, “Acid-Washed Graphitized Carbon Black for Gas Chromatography”. Anal. Chem., 1980, 52, (8), pp 1345-1350.

Supelco Inc., Supelco Reporter, Vol 1, No. 1, 1982, pp 6-7.

Supelco Inc., Product Bulletin #790C.

Location of Validation Package.

Quality System Files

Required Training, Certification and Re-certification.

1. In-house training by a certified chemist in GC and chemstation operation. Training on GC (in-house or vendor provided).

2. Periodically, chemists are re-tested for competency (e.g., every 5 years) and/or given proficiency testing.

Revision History.

Revision 4 – changes as a result of a document review to clarify and harmonize units used in the test method – 11/14/2008

Revision 5 – Change reporting of Propylene Glycol to 2 decimal places from 1 – 9/1/2009

Revision 6 – changed DL, QL and linear range units to match reporting units; added values to DL, QL and linear range to cover both DS and wine units.

Revision 7 – changes to calibrant levels used; changes to LOD and LOQ, edits for clarity and to better reflect lab practices; addition of Sources of Uncertainty; addition of what to try for precision QC failures (Quality control section)

NOTE: Revision 7 had errors in units in the standard concentrations. This has been revised and issued prior to implementation date. 10/29/2014

Revision 8 – addition of second source solution instructions; added instructions to Quality Control; added Blanks to the procedure; added requirement for running a second source check, as well as bracketing samples with a second source check to the procedure.