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.
3. ***Distilled spirit products containing >10% solids are diluted (or distilled) prior to analysis. NOTE: Propylene glycol and glycerol cannot be determined after distillation.***
4. Liqueur products are distilled prior to analysis. NOTE: 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/1***00L)	***5.9-20000 mg/L (0.59-2000 g/100L)***	None
Methanol	0.000***4*** %	0.00***5*** %	***0.015-1.5*** %	***0.01-20%***	None
n-Propanol	0.***5*** mg/L (0.0***5*** g/100L)	***1.6*** mg/L (0.***16*** g/100L)	***3-300 m***g/***L(0.3-30g/***100L)	***1.6-10000 mg/L (0.16-1000 g/100L)***	None
iso-Butanol	0.***5*** mg/L (0.0***5*** 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 (0.0***3*** g/100L)	0.***9*** mg/L (0.0***9*** g/100L)	***3-300 m***g/***L(0.3-30g***/100L)	***1.25-2000 mg/L (0.125 – 200 g/100L)***	None
Amyl Alcohol	0.***3*** mg/L (0.0***3*** g/100L)	***1.0*** mg/L (0.***10*** g/100L)	***12-1200 m***g/***L(1.2-120g***/100L)	***2-40000 mg/L (0.2-4000 g/100L)***	None
Acetic Acid **	0.00***1*** g/100mL	0.00***32*** g/100mL	0.0***1***-0.2***0*** g/100mL	***0.002-0.2 g/100mL***	Furfural
Benzaldehyde	0.00***1***4 g/100mL	0.00***46*** g/100mL	0.0***1***-0.2***0*** g/100mL	***0.002-0.2g/100mL***	None
Propylene Glycol	0.00***10***% by Vol	0.00***33*** % by Vol	0.0***1***-0.2***0*** % by Vol	***0.002-0.2% by Vol***	None
Glycerol	0.00***6*** % by Vol	0.0***19*** % by Vol	0.0***5***-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, 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 and working standards: *See Table below*

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%
d. 4.00 g ***active*** amyl alcohol ± 1.0%
e. 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. Q.S. with 40% ethanol by volume.

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

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

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

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

Analyte	*Stock Std. ppm*	*FO4 ppm*	*FO3 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 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.

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

3. Inject samples.

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

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. Re-run a ***second source*** middle standard(s) ***at a concentration within the range of calibrants*** (***for example FO3*** and/or NBA std 2) at least once every 10 samples as a control to check for drift. If the standard differs by >1***5***% of the expected value, repeat the previous 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.

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