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Capillary GC Analysis of Fusel Oils and Other Components of Interest

Scope and Application

Methanol, acetaldehyde, ethyl acetate and fusel oils (FO) 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. Liqueur products are distilled prior to analysis.
4. 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 $\leq$ 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 $\leq$ 28

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

Propylene Glycol For NBP's, $\pm$ 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, $\pm$ 5% of the stated value. The finished alcohol beverage may not contain more than 0.15% acetic acid (21 CFR 184.1005).

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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	0.002-0.2 g/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.

Supplemental Documents

1. SSD:QPD:3100 Laboratory Quality Control
2. WG:SSD:1040:004 Beer and Wine Degassing Procedure
3. SSD:TM:102 Ethanol Determination by SG

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Equipment

Instrumentation:

GC: Agilent 8890 or equivalent
Column: DB-WAXetr, 30m x 0.53mm x 1µm film thickness or equivalent
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
ALS Syringe: ALS syringe, 10 µL, fixed needle, 23/42/cone 23 g straight needle
(part number 9301-0725, or equivalent)

Glassware and Supplies:

Class A pipets / Micropipettes
Class A volumetric flasks

Reagent and Sample Preparation and Handling

Reagents:

Note: All chemicals for standards are 99.0+% pure.

200 Proof Ethanol

Deionized Water

Ethyl Acetate

Methanol

n-Propanol

iso-Butanol

n-Butanol

2-methyl-1-butanol (active Amyl alcohol)

Acetic Acid

Benzaldehyde

Propylene Glycol

Glycerol

Acetaldehyde

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Preparation of FO stock, working, and second source standards:

1. FO Stock Standard

Prepare the stock standard to the concentrations listed below within $\pm 1\%$ of target mass, then Q.S. with $>95\%$ ethanol by volume. The stock solution is stable in the refrigerator for up to 12 months.

Analyte	Stock Concentration
Ethyl Acetate	30,000 ppm
Methanol	50,000 ppm (50.0% by vol)
n-Propanol	10,000 ppm
iso-Butanol	20,000 ppm
n-Butanol	10,000 ppm
Amyl Alcohol	40,000 ppm

For example, if preparing 100 mL stock standard the following table of masses and volume could be used. It is recommended to start in the order listed below to minimize evaporation of analytes.

Analyte	Example Target Mass/Volume	Example $\pm 1\%$ Target Mass
Amyl Alcohol	4.00 g	3.9600 – 4.0400 g
n-Butanol	1.00 g	0.9900 – 1.0100 g
iso-Butanol	2.00 g	1.9800 – 2.0200 g
n-Propanol	1.00 g	0.9900 – 1.0100 g
Ethyl Acetate	3.00 g	2.9700 – 3.0300 g
Methanol	50.00 mL	Use a 50 mL Class A Volumetric Pipette

2. FO Working Standards

Prepare FO working standards by transferring stock or diluted standards to the below listed concentrations using class A volumetric pipettes or positive displacement pipettes. The standards may be stored in the refrigerator for up to 3 months.

Analyte	Level 4 [ppm]	Level 3 [ppm]	Level 2 [ppm]	Level 1 [ppm]
Ethyl Acetate	900	150	22.5	9
Methanol	15,000 (1.5% by vol)	2,500 (0.25% by vol)	375 (0.037% by vol)	150 (0.015% by vol)
n-Propanol	300	50	7.5	3
iso-Butanol	600	100	15	6
n-Butanol	300	50	7.5	3
Amyl Alcohol	1200	200	30	12

For example, if preparing 200 mL working standards the following table could be used.

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Working Standard	Example Preparation
FO Level 4	Transfer 6 mL standard stock solution into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.
FO Level 3	Transfer 1 mL standard stock solution into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.
FO Level 2	Transfer 5 mL Level 4 into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume.
FO Level 1	Transfer 2 mL Level 4 into 200 mL volumetric flask or equivalent concentration. Q.S. with 40% ethanol by volume

3. FO Second Source

Prepare FO second source stock and working standard in the same manner as FO Stock Standard and Level 3 Working Standard.

Preparation for NBA stock and working standards:

1. NBA Stock Standard

Prepare an NBA standard stock solution to the concentrations listed below within $\pm 1\%$ of target mass, then Q.S. to volume with 40% ethanol. Store stock solution in the refrigerator for up to 12 months.

Analyte	Stock Concentration
Acetic Acid	4,000 ppm or 0.2 g/100mL
Benzaldehyde	4,000 ppm or 0.2 g/100mL
Propylene Glycol	4,140 ppm or 0.2% by vol
Glycerol	25,000 ppm or 1.0% by vol

For example, if preparing 1 L stock standard the following table of masses could be used.

Analyte	Example Target Mass	Example $\pm 1\%$ Target Mass
Acetic Acid	2.00 g	1.9800 – 2.0200 g
Benzaldehyde	2.00 g	1.9800 – 2.0200 g
Propylene Glycol	2.07 g	2.0493 – 2.0907 g
Glycerol	12.50 g	12.3750 – 12.6250 g

2. NBA Working Standards

Prepare an NBA working standards to the concentrations listed below, then Q.S. to volume with deionized water. Working standards are prepared daily.

Analyte	Level 2	Level 1
Acetic Acid (g/100mL)	0.1	0.01
Benzaldehyde (g/100mL)	0.1	0.01
Propylene Glycol (% by vol)	0.1	0.01
Glycerol (% by vol)	0.5	0.05

For example, if preparing 10 mL working standards the following table could be used.

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Working Standard	Example Preparation
NBA Level 1	Transfer 0.5 mL standard stock solution into 10 mL volumetric flask or equivalent concentration. Q.S. with deionized water.
NBA Level 2	Transfer 5 mL standard stock solution into 10 mL volumetric flask or equivalent concentration. Q.S. with deionized water.

3. NBA Second Source

Prepare an NBA second source solution to the concentrations listed below within $\pm 1\%$ of target mass, then Q.S. to volume with 40% ethanol. Store second source solution in the refrigerator for up to 12 months.

Analyte	Second Source Concentration
Acetic Acid	0.1 g/100mL
Benzaldehyde	0.1 g/100mL
Propylene Glycol	0.01 % by vol
Glycerol	0.5 % by vol

For example, if preparing 500 mL stock standard the following table of masses could be used.

Analyte	Example Target Mass	Example $\pm 1\%$ Target Mass
Acetic Acid	1.00 g	0.9900 – 1.0100 g
Benzaldehyde	1.00 g	0.9900 – 1.0100 g
Propylene Glycol	1.035 g	1.0247 – 1.0454 g
Glycerol	6.25 g	6.1875 – 6.3125 g

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.

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Procedures

1. Check to see if **neat** samples (e.g., NBA, enforcement) were run with the current liner. If so, replace the liner.
2. Inject washes, blanks, samples, and second source (QC) using the following recommended sequence template:
 - Wash (e.g., 40% ethanol)
 - Blank (e.g., 40% ethanol)
 - Calibration Standards (FO level 1-4, NBA level 1-3, or NBA Level 1-3 followed by FO level 1-4)
 - Blank
 - QC check (2nd source)
 - Two LCS
 - Sample(s)
 - QC check (2nd source)
3. Confirm all washes, blanks, calibration curves, LCS samples, and QC checks pass the limits specified in the Quality Control section.

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

4. Report results as described in the Reporting Results section.

Quality Control

1. The correlation coefficient (R^2) of the analytes of interest is to be >0.99 . If R^2 is <0.99 , change the liner and rerun injections. If the R^2 is still <0.99 , re-run using fresh working standards. If the R^2 remains out of spec, contact the instrument team.
2. Run 2 LCS samples for accuracy and precision. The values for accuracy and precision are to be within the prescribed limits.
3. Run a QC at least once every 10 samples (including the LCS). The QC check is to be within 15% of the expected value. If any second source analytes exceed the tolerance limits, inspect the liner and syringe and clean or replace as necessary. Remake second standard(s) if results exceed the tolerance limits on more than one instrument.
4. Wash injections are allowed to contain carryover $>LOQ$. Inject a wash after potentially "dirty" samples (i.e., high solids, high contaminants) to assure the system cleans out properly prior to the next injection.
5. Blank injections are acceptable if results are $<LOQ$ for all analytes being reported. There must be a blank at the beginning of a sequence and after the highest calibration point. If unacceptable, do not report affected results and inspect liner, syringe/plunger and replace or clean if necessary.
6. Violative results are confirmed as follows:
Acetic Acid - confirm using a TTB Official method (SSD:TM:502 or SSD:TM:503).

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7. For precision quality control failures, inspect the liner and syringe 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	2 decimals	X.xx
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 SDS 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.

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

References

Official Methods of Analysis (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, **Method 968.09** (*Alcohols (Higher) and Ethyl Acetate in Distilled Liquors*). www.eoma.aoac.org [accessed on March 25, 2021]

Note: Similarities – Method 968.09 is the basis for SSD:TM:200 to separate higher alcohols in distilled liquors using GC-FID; Differences – Method 968.09 has been modified with different standard mixtures, and instrumentation/column

Official Methods of Analysis (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, **Method 972.11** (*Methanol in Distilled Liquors*). www.eoma.aoac.org [accessed on March 25, 2021]

Note: Similarities – Method 972.11 prepared methanol standard solutions in 40% ethanol without the use of internal standard; Differences – Method 972.11 differed in the standard mixture, quantifying using peak height instead of peak area, and GC column and operating parameters

Martin, G.E., et al. (1981) *J. AOAC Int.* **64**, 1, 186-190. Title: *Gas-Liquid Chromatographic Determination of Congeners in Alcoholic Products with Confirmation by Gas Chromatography/Mass Spectrometry*.

Note: Similarities – standard mixture in 40% ethanol; Differences – SSD:TM:200 was modified to updated instrumentation/column

Kelly, J., et al. (1999) *J. AOAC Int.* **82**, 6, 1375-1388. Title: *Gas Chromatographic Determination of Volatile Congeners in Spirit Drinks: Interlaboratory Study*.

Note: Similarities – calculation using peak area; Differences – differed from SSD:TM:200 with use of internal standard, and modification of instrument parameters/column dimension

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.

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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.

Revision 9 – additions to procedure for quality items; differentiation between 'blank' and 'wash' injection; added instruction to quality control for second source and LCS; update references listed.

Revision 10 – Updated reporting requirements for Benzaldehyde to 2 decimal places; added example preparations to stock and working standards; updated chemicals to Reagent and Sample Preparation and Handling; added syringe specifications to Equipment. Updated Procedures and Quality Control section. Standardized Blanks, Washes, and added example sequence table.