GC-FID for the Analysis of Ethanol and Propylene Glycol in Liquid Nonbeverage Products

Scope and Application

The objective of this method is to determine the alcohol by volume (% ABV) and propylene glycol by volume (% v/v) of liquid nonbeverage samples using GC-FID. This method may not be applicable to samples containing n-propanol as it is used as the internal standard. This method has been determined to be fit for the purpose of determining the alcohol content by volume (between ~0.4 and 95 %ABV) and PG content (~1 to 98 %v/v) in liquid nonbeverage products (including flavors/flavorings, botanical extracts, salted cooking wines, etc.)

NOTE: The tolerance table used for nonbeverage formulas is provided in Appendix 1. Tolerances for Item #10 refers to Item 10 on TTB Form 5154.1, Alcohol Content by Volume of Finished Product (or the equivalent field in Formulas Online)

Levels and Limitations

This method is fit for liquid nonbeverage samples that are miscible in methanol since methanol is the solvent used for dilution. Products with less than 0.5 % ABV are considered non-alcoholic. Therefore, the method has a target range for ethanol of ~0.4 % ABV to 95 % ABV and the range of propylene glycol is ~1-98 % v/v. If the propylene glycol content is less than ~1 % v/v, then SSD:TM:200 (Capillary GC Analysis of Fusel Oils and Other Components of Interest) can be used to get an exact concentration if needed.

This method may not be applicable to samples that contain n-propanol, as n-propanol is used as the internal standard. The ethanol used in the validation contains a very small amount of n-propanol as an impurity and the methanol used in the validation contains a very small amount of ethanol as an impurity. The n-propanol impurity is so small that the effect on the quantitation is negligible. The ethanol contamination does not impact the quality of the results, as the methanol is added in the same fashion to all standards and samples.

Furthermore, isopropanol elutes closely before ethanol and therefore samples with high concentrations of isopropanol may affect the quantitation of the ethanol peak. At ~30 % v/v isopropanol and ~30 % v/v ethanol in water, the two peaks co-elute at a level which may affect the quantitation. Furthermore, if one of the two compounds is at a very high level (~80 % v/v) and the other is moderately high (~20 %v/v), then co-elution could also affect the quantitation. If high levels of ethanol and isopropanol are expected in a sample, then the analyst should verify that the ethanol peak has integrated accurately.

Analyte	Detection Limit	Quantitation Limit	Linear Range	Interferences
Ethanol	0.025 % v/v	0.075 % v/v	0.4 – 95 % v/v	Isopropanol*
Propylene Glycol	0.05 % v/v	0.15 % v/v	1 – 98 % v/v	

*Please see last paragraph of Levels and Limitations for details of interference

Supplemental Documents

1. SSD:TM:200 Capillary GC Analysis of Fusel Oils and Other Components of Interest
2. SSD:TM:103 Ethanol, Specific Gravity and Refractive Index Determination in Nonbeverage Products
3. NPL:WG:103 Hamilton Microlab 600 Basic Autodiluter
4. NPL:Form:218-1 SSD:TM:217 Standards Calculations Worksheet
5. NPL:Form:218-2 SSD:TM:217 QC Calculations Worksheet

Equipment

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

Instrumentation and Run Conditions:

GC:	Agilent 7890B with 7693 Autosampler and OpenLab CDS Chemstation Software
Column:	DB-WAXETR, 30 m x 0.530 mm x 1 µm
Carrier Gas:	Hydrogen, constant flow, 5.9 mL/min
Temperature Program:	40 °C initial, hold 5 min, ramp at 10 °C/min to 150°C, 35 °C/min to 215 °C hold 2.5 min.
Injector:	220 °C, 5:1 split ratio
Injection Volume:	1 µL
Rinse Solvent:	Wash A – DI Water; Wash B – 100 % Methanol
Detector:	FID 250 °C, hydrogen flow 40 mL/min, air flow 450 mL/min, 25 mL/min nitrogen makeup flow

Autodiluter Hamilton Microlab 600 Autodiluter. Please refer to the Autodiluter logbook and working guideline (NPL:WG:103) for information about the proper use of the autodiluter.

Densitometer, Anton Paar DMA 4500M, reporting specific gravity to 5 decimal places (+/- 5 in the last decimal place or better), temperature controlled 20.00 +/- 0.02 °C. Please refer to laboratory guidelines, instrument manuals, and SSD:TM:103 for more information about the proper use of a DMA.

Analytical Balance. An analytical balance (Sartorius 1602 MP8-1) will be used in the preparation of standards. Such a balance should read to at least 4 decimal places.

Glassware and Supplies: (Specific vendors and product numbers are listed for convenience. Equivalent products may be used.)

Class A volumetric flasks, various volumes as needed
Class A volumetric pipets or autopipettors, various sizes, as needed
Gas chromatograph vials and caps, Fisher Scientific Part # 06-406-19E and 06-406-19B, respectively.
Glass storage bottles with tight-fitting lids, 100 mL – 1 L, as needed, Fisher part number 02-911-353.
Glass Pasteur pipettes (for weighing), if desired
Graduated cylinders, various volumes as needed
Kimwipes, Kimberly Clark Delicate Task Wipes, Fisher part number 06-666A
Parafilm, Curwood Laboratory Wrapping Film, Fisher part number 13-374-10

Reagent and Sample Preparation and Handling

Reagents: (Specific vendors and product numbers are listed for convenience. Equivalent products may be used.)

Deionized water
Ethanol, Pharmco-Aaper, 200 proof, anhydrous, absolute, Part # 111000200
Second Source, Fisher part number AC615101000
Methanol, ACS/HPLC grade, Fisher part number A452-4
n-Propanol, > 99.5 %, Fisher part number BP1130
Propylene Glycol, 99.5 %, Sigma Aldrich Catalog # W294004
Second Source, Fisher part number P355-1

Preparation of Internal Standard Solution (Diluent):

A solution of 2.3 % v/v n-propanol is prepared by transferring 23 mL of n-propanol to a 1000 mL Class A volumetric flask, diluting to the mark with methanol, and inverting to mix. The solution is stable when stored capped and at room temperature in a clear glass container for at least one year. Please note that volumes may be adjusted as desired; however, ensure that the final concentration remains approximately 2.3 % v/v.

Preparation of Working Standards and QC Standards:

Use the preparation scheme outlined below for making the working standards and QC standards. Be sure to record the weight of propylene glycol and weight of the final

solution as these values are needed to calculate the ethanol and propylene glycol content. Please note that weights and volumes may be adjusted as desired; however, ensure that the final concentrations remain close to the desired levels listed below. Determine the precise alcohol by volume and density of the 96 % Ethanol Water Mixture and Standard #1 with a densitometer (refer to SSD:TM:103). The concentration of ethanol in the solutions may be adjusted after initial DMA analysis to achieve more "ideal" levels, if desired (be sure to re-analyze on the DMA to establish the final concentration of the solution). For Standards # 2 – 7, determine the precise density of each solution using a densitometer, this will be used to determine % ABV and % v/v PG of the final solution. The calculations used for the standards are described in the Calculations section of this test method. A worksheet to perform the calculations for the standards is listed under the Supplemental Documents section. A second source ethanol and propylene glycol should be used to prepare QCA and QCB when possible.

Store these working solutions in tightly sealed bottles (the use of parafilm around the cap is recommended) in the refrigerator for up to 1 year.

NOTE: Be sure to allow mixtures of ethanol and water to equilibrate to room temperature before bringing to volume, if preparing by volume.

96% Ethanol Water Mixture (EW Mix):
96 mL ethanol + 4 mL DI water

Standard 1 (0.4%): 0.4 mL ethanol + 99.6 mL DI water

Standard 2 (~1.0% EtOH – 99.0% PG):
1.0 mL EW Mix + 99.0 mL PG

Standard 3 (~9.0% EtOH – 91.0% PG):
9.0 mL EW Mix + 91.0 mL PG

Standard 4 (~25.0% EtOH – 75.0% PG):
25.0 mL EW Mix + 75.0 mL PG

Standard 5 (~50.0% EtOH – 50.0% PG):
50.0 mL EW Mix + 50.0 mL PG

Standard 6 (~75.0% EtOH – 25.0% PG):
75.0 mL EW Mix + 25.0 mL PG

Standard 7 (~95.0% EtOH – 1.0% PG):
99.0 mL EW Mix + 1.0 mL PG

QCA (~3.0% EtOH, 5.0% PG, and 92.0% DI Water):
3.0 mL EW Mix + 5.0 mL PG + 92.0 mL DI Water

QCB (~60.0% EtOH – 40.0% PG):
60 mL EW Mix + 40.0 mL PG

Preparation of Laboratory Control Sample (LCS):

Two LCSs are used for this method. Both are made using a sample or mixture of samples with a good representation of a flavor mixture. LCS 1 should have a low ethanol concentration and middle/high propylene glycol concentration. LCS 2 should have a middle/high ethanol concentration and low propylene glycol concentration. The actual values for ethanol and propylene glycol concentrations should be determined using consensus values (preferably multiple analysts, over multiple days and using multiple instruments). The expiration of the LCS will be 2 years from the date prepared.

Preparation of Samples:

Each sample should be shaken to uniformly mix before diluting in the diluent. Samples which are expected to have an alcohol content greater than the highest standard should be diluted using methanol prior to diluting in the diluent.

Procedures

1. Allow standards, QCs and LCSs to equilibrate to room temperature (roughly 65-80 °F).
2. Prime the autodiluter by flushing the diluent solution through with 10 prime cycles.
    a. Ensure there is enough diluent in the container for all of the analyses for the week. If the container diluent runs out or is changed, the calibration standards and QCs must be prepared again using the new diluent.
3. Load the 1 to 10 dilution custom method.
    a. The 1 to 10 dilution custom method draws up diluent (675 µL) then waits for analyst intervention. When the analyst depresses the button, sample is drawn up (75 µL). After the sample the probe is wiped with a Kimwipe to ensure that no extra sample is being introduced into the final dilution. After another press of the button, an air gap is drawn up. Next, the dilution is dispensed into the autosampler vial with a final press of the button (750 µL total). The probe is touched to the side of the vial to ensure that all drops of desired diluent were delivered to the vial. Cap the vials, then shake well (or vortex) to mix. The sample probe is rinsed with DI water between standards/samples by making two “blank” dilutions into the waste container. The sample probe should also be rinsed with each standard/sample twice before dispensing it into the autosampler vial.
4. Prepare all standards, QCs, LCSs, samples using the 1 to 10 dilution custom method. Alternatively, manual dilution with pipets can be used to prepare the standards, QCs, LCSs and samples.
    a. A blank (DI water prepared in the diluent like a sample) should be prepared.
    b. The calibration standards and QCs can be prepared in GC vials and left on the GC for 1 week. They should be run each day along with the freshly prepared LCSs and samples.
    c. LCS1 and LCS2 should be prepared in duplicate each time samples are prepared and run during the sequence.
5. Standards, QCs, LCSs and samples are analyzed using the GC with the parameters listed above.

6. Calibrate using area, internal standard, weighted linear curve (in Openlab Chemstation it is denoted as Linear (Amnt)). The calibration curve does not use nor is it forced through zero. The correlation coefficient should be ≥0.999.

Quality Control

1. The correlation coefficient of the standards should be ≥0.999. Up to 1 data point may be removed from the calibration as long as all of the samples analyzed fall within the range of the remaining calibration standards. If the correlation coefficient of the standards is not ≥0.999, replace liner and/or septum and re-run. If the correlation coefficient still is not ≥0.999, then re-run using freshly made standards. If the correlation coefficient still is not ≥0.999, then contact the co-principal analyst.

2. The method uses two QCs which should be run to bracket every 10 samples during the analysis. The QCs can be prepared with the calibration standards and are good for one week stored in the sealed GC autosampler vial.

3. The method uses 2 LCSs which should each be prepared in duplicate whenever samples are prepared and treated as samples.

4. A blank (water prepared as a sample in the diluent) should be analyzed to ensure no contamination.

5. Any LCS failure requires the initiation of a CAR (Refer to SSD:QPD:310 for more information). If one of the control samples (LCS or QC) falls outside the accuracy range then re-prepare the one that failed and re-run the sequence. If all quality data passes then report the data; if any of the quality data fails then tag instrument out of service and initiate a CAR. If more than one of the control samples (LCS or QC) falls outside the accuracy range then re-prepare all of control samples and regular samples and re-run. If all quality data passes then report the data; if any of the quality data fails then tag instrument out of service and initiate a CAR. **NOTE**: In the case of a QC failure, all samples bracketed by passing QCs are reportable and do not have to be re-run.

Sources of Uncertainty

1. Weighing errors for standards
2. Error in using autodiluter to add diluent to samples
3. Coelution of matrix component with an analyte
4. Dirty injection liner
5. Problem with GC syringe (e.g., dirty syringe or bad plunger)

Calculations

The following calculations are automatically performed using NPL:Form:218-1 and NPL:Form:218-2 once all collected data is entered into the correct fields.
For Standards #2 - 7, the weight of PG in the standard is divided by the density of PG (1.036 g/mL) which yields the volume of PG in the standard. Then, the weight of the total

standard is divided by the density of the standard obtained from the DMA yielding the total volume of standard. The % v/v of PG in Standards #2 - 7 is calculated by dividing the volume of PG by the total volume of the standard and multiplying by 100.

For determining the ethanol content by volume of each standard, the weight of the EW Mix in the standard is calculated by subtracting the weight of PG from the total weight of the standard. The weight of EW Mix in the standard is then divided by the density of the EW Mix to yield the volume of the EW Mix in the standard. The % alcohol by volume of the EW Mix (from the DMA analysis; decimal equivalent) is then multiplied by the volume of the EW Mix in the standard to yield the volume of absolute ethanol in the standard. The total volume of the standard is obtained by dividing the total weight of the standard by the density of the standard. The % alcohol by volume is then calculated by dividing the volume of absolute ethanol in the standard by the total volume of the standard and multiplying by 100.

The GC is operated using internal standard mode with calculations using peak areas. The GC data analysis software will perform all calculations for sample concentrations.

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
Ethanol	Liquid Nonbeverage Samples	% v/v	1 decimal	***X.x***
Propylene Glycol	Liquid Nonbeverage Samples	% v/v	1 decimal	***X.x***

Safety Notes

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

High proof alcohol products are flammable. Ethanol burns with an almost invisible blue flame. Use extreme caution when working with alcohol products.

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 hydrogen leak sensor to automatically shut down the GC, this method shall not be used.

Waste produced during preparation of standards and samples can be disposed of using the flavor waste stream. Used GC vials can be disposed of using the GC/LC vial waste stream.

References

None

Required Training, Certification and Re-certification

1. Receive in house 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 proficiency test.

Revision History

Rev. 1 – initial revision

Rev. 2 – Updated injector split ratio; Added a second source for reagents; Added statement to use a second source when available; Added statement that manual pipetting may be performed; Added statement that one calibration point may be removed, if all samples fall within the remaining calibration curve.

Rev. 3 - Added requirement to procedures requiring analyst to ensure there is enough diluent in container at start of week, and requiring calibration standards and QC be re-prepped when using new diluent.

Appendix 1

Tolerances for Item #10

Alcohol Percentage	Simple Mixtures	Processes Other Than Simple Mixtures *
> 95-100	93-100	91-100
>90-95	88-95	86-95
>80-90	+/- 3.5	+/- 4.5
>70-80	+/-3.0	+/- 4.0
>60-70	+/- 2.5	+/- 3.5
>40-60	+/- 2.0	+/- 3.0
>20-40	+/- 1.5	+/- 2.5
>1-20	+/-1.0	+/- 2.0
0.5-1.0	+/-0.5	+/- 0.5

Please note that the percentages are absolute. For example, at 18% alcohol by volume, the value in #10 for a simple mixture should be 18 +/- 1.0. In addition, the value in box # 10 cannot be less than zero for any range.

* Due to the inherent variability associated with processing botanical materials, we allow a range of +/- 5 for dietary supplements. However, the value in box #10 cannot exceed the amount of alcohol theoretically present, and cannot be less than zero.

Example Chromatograms: