Alcoholic Beverage Fusel Alcohol Testing with Static Headspace

Application Note

Abstract

A static headspace method was developed using Teledyne Tekmar automated headspace vial samplers (Figure 1 and Figure 2) to meet the method requirements of the Alcohol and Tobacco Tax and Trade Bureau of the US Department of the Treasury (TTB) method SSD: TM:200[1] for testing fusel alcohols in alcoholic beverages.

Figure 1 Versa Automated Headspace Vial Sampler

Figure 2 HT3 Static and Dynamic Headspace Analyzer

Introduction

Fusel alcohols are a common byproduct of the fermentation process, but abnormal quantities or types can indicate alcoholic beverage adulteration. Method SSD: TM:200[1] is a direct injection method for testing these alcohols. The method states that NonBeverage Alcohol (NBA) products, not miscible with water, cannot be analyzed by this method. Additionally, NBA products and distilled spirits with greater than 10% solids must be diluted prior to analysis.

The direct injection technique transfers all compounds in the sample into the inlet and could potentially contaminate the inlet liner, requiring frequent changes. To compensate for this contamination, the quality control section of SSD: TM:200 indicates that when the correlation coefficient drops below 0.99, the liner should be changed as a corrective action. In addition to liner contamination, thermally labile compounds such as sugars can break down, leading to interfering peaks that hinder analysis.

A Teledyne Tekmar static headspace vial sampler will be used to develop a static headspace method to test for fusel oil following the TTB method. By using static headspace, only the volatile component will be transferred to the GC, leaving the nonvolatile components that can foul an injection liner in the headspace vial.

Experimental - Instrument Conditions

For this study, the HT3 was coupled to a GC/FID system. Helium was used as the GC carrier gas and FID makeup gases, as well as the HT3 pressurization gas. The Method Optimization Mode (M.O.M.) feature of the HT3's TekLink™ software was utilized to develop the sample temperature study.

The GC was equipped with a ZB-624 column whose dimensions are 30 m length x 0.32 mm ID x 1.8 µm film thickness. The GC/FID parameters are shown in Table I and Table II. Table III shows the HT3 instrument parameters.

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Table I GC Parameters	
Column	624-type, 30 m x 0.32 mm ID x 1.8 µm
Oven Program:	35 °C for 5 min, 10 °C/min to 260 °C with a 2.5 min final hold, Run time 30 min
Inlet:	250 °C
Column Flow	2 mL/min, Average Velocity 33 cm/sec
Gas:	Helium
Split:	20:1
Pressure:	9.23 psi

Table II FID Parameters	
FID Temp:	250 °C
Hydrogen:	35 mL/min
Air:	300 mL/min
Makeup Gas:	Helium
Makeup Flow:	30 mL/min

Table III HT3 Instrument Parameters

Variable	Value	Variable	Value
Constant Heat Time	On	Mixing Time	5.00 min
GC Cycle Time	39.00 min	Mixing Level	Level 5
Valve Oven Temp	105 °C	Mixer Stabilize Time	0.50 min
Transfer Line Temp	120 °C	Pressurize	15 PSIG
Standby Flow Rate	50 mL/min	Pressurize Time	1.00 min
Platen/Sample Temp	M.O.M.	Pressurize Equil Time	0.20 min
Platen Temp Equil Time	0.10 min	Loop Fill Pressure	12 PSIG
Sample Equil Time	40.00 min	Loop Fill Time	0.50 min
Mixer	Off	Inject Time	2.00 min

(Parameters highlighted in yellow were not used)

Standard and Sample Preparation

The fusel oil stock standard was prepared following the SSD: TM:200 procedure. Working standards were prepared to encompass the range listed in the procedure (refer to Table IV). The two amyl alcohols, isoamyl alcohol (3-methyl-1-butanol) and active amyl alcohol (2-methyl-1-butanol), were used to prepare the total amyl alcohol concentration listed in the procedure. All working standards were prepared by diluting the volume of stock standard to 250 mL with water.

Table IV Working Standard Concentrations

Compound	10 mL	5 mL	2 mL	1 mL	0.2 mL
Ethanol	4% v/v	2% v/v	0.8% v/v	0.4% v/v	0.08% v/v
Ethyl Acetate	800 mg/L	400 mg/L	160 mg/L	80 mg/L	16 mg/L
Methanol	0.8% v/v	0.4% v/v	0.16% v/v	0.08% v/v	0.016% v/v
n-Propanol	400 mg/L	200 mg/L	80 mg/L	40 mg/L	8 mg/L
Isobutanol	800 mg/L	400 mg/L	160 mg/L	80 mg/L	16 mg/L
n-Butanol	40 mg/L	20 mg/L	8 mg/L	4 mg/L	0.8 mg/L
Isoamyl Alcohol	800 mg/L	400 mg/L	160 mg/L	80 mg/L	16 mg/L
Active Amyl Alcohol	800 mg/L	400 mg/L	160 mg/L	80 mg/L	16 mg/L

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Samples were prepared by measuring 10 mL of the working standard or blank water into 22 mL headspace vials. The TekLink Method Optimization Mode (M.O.M.) software feature was used to create a series of headspace methods varying the sample/platen temperature from 40 °C to 90 °C in 10 °C increments.

Results and Discussion

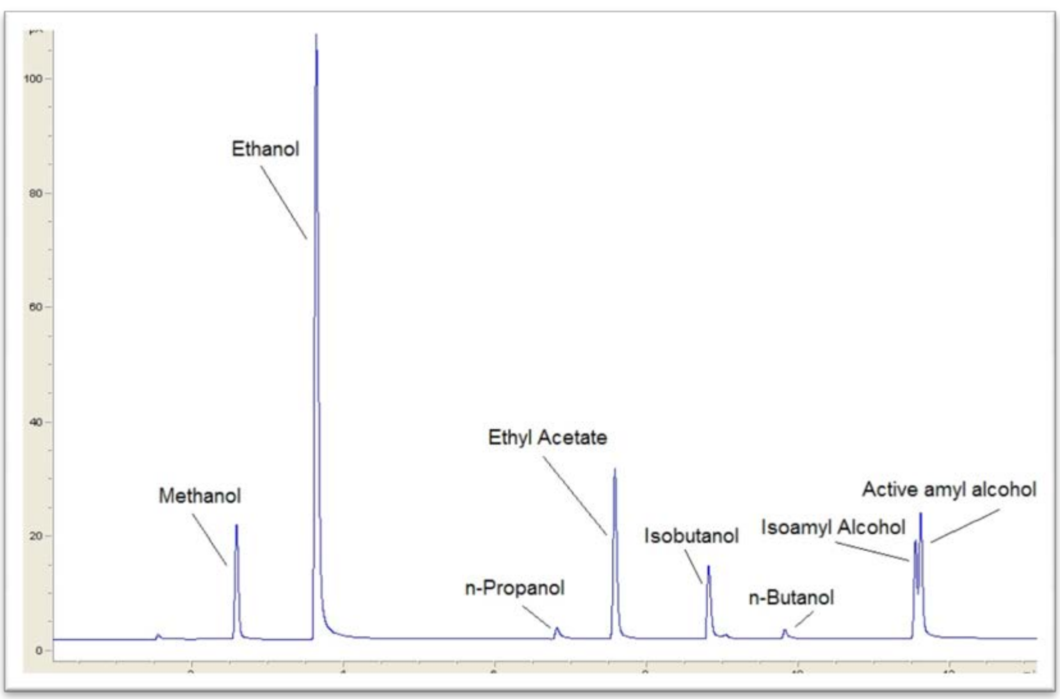
The TTB procedure has a quality control requirement that the correlation coefficient (r^2), calculated by external standard method, is to be greater than 0.99 for the compounds in the fusel oil standards. The correlation coefficient and the Response factor percent Relative Standard Deviation (Rf %RSD) was plotted for each compound at the various platen temperatures.

The results showed that a sample temperature of 70 °C would best meet the requirements of the TTB method with an r^2 value of greater than 0.99 and an Rf %RSD of less than 10%. Table V shows the r^2 and Rf values for the fusel oils at 70 °C. A chromatogram of the lowest level standard, 0.2 mL, is shown in Figure 3.

Table V Results

Compound	Concentration Range	r^2	Rf
Methanol	0.016% to 0.8% v/v	0.9981	5.05
Ethanol	0.08% to 4% v/v	0.9984	4.02
n-Propanol	8 to 400 mg/L	0.9990	7.70
Ethyl Acetate	16 to 800 mg/L	0.9995	3.50
Isobutanol	16 to 800 mg/L	0.9999	1.01
n-Butanol	0.8 to 40 mg/L	0.9991	6.72
Isoamyl Alcohol	16 to 800 mg/L	0.9996	3.36
Active Amyl Alcohol	16 to 800 mg/L	0.9997	8.50

Figure 3 Chromatogram of the 0.2 Level Standard at 70 °C



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Conclusions

The current TTB method, SSD: TM:200 for analyzing fusel oils in beer, wine, nonbeverage product and moonshine, was easily converted to a static headspace method. The static headspace method was developed with the HT3 utilizing the TekLink M.O.M. software feature that simplified selecting the best temperature for analysis of the listed fusel oils.

The static headspace sample temperature of 70 °C provided excellent correlation coefficient and Response factor percent Relative Standard Deviation (Rf %RSD) value for all of the fusel oil compounds. The SSD: TM:200 criteria of r^2 value greater than 0.99 was met at this temperature.

The headspace method, unlike a direct injection technique, transfers only the volatile components of the sample. The semivolatile solids and other nonvolatile compounds, such as sugars that can quickly contaminate a GC inlet liner and column, remain in the headspace vial. Benefits are two-fold, reducing a lab's budget by minimizing frequent column and inlet liner changes, as well as increasing instrument uptime.

The HT3 or Versa automated headspace vial samplers can benefit beverage laboratories by increasing throughput and testing more samples. Additionally, the HT3 has a 60 sample autosampler while the Versa has a 20 sample autosampler to match the needs of large production beverage companies down to small microbreweries.

References

1) Capillary GC Analysis of Fusel Oils and Other Components of Interest, US Department of Treasury, Alcohol and Tobacco Tax and Trade Bureau, http://www.ttb.gov/ssd/pdf/tm200.pdf

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