Headspace GC-MS Determination of Ethanol in Nonbeverage Alcohol Products

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Abstract

As part of the U.S. Department of Treasury the Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for regulating the alcohol beverage industry in the United States and collecting revenue (excise tax). One component of TTB's jurisdiction is the regulation of nonbeverage alcohol (NBA) products. These products include medicines and medicinal preparations; food products; flavors and flavoring extracts; and perfumes manufactured using taxpaid distilled spirits and which are unfit for beverage purposes. Manufacturers may claim drawback on the taxes paid for eligible spirits which were used in the production of approved NBA products.

Determination of alcohol content is a necessary step in the evaluation of NBA products submitted for drawback approval, but some of these products (e.g. sauces, syrups, gels, cakes, ice creams) are not amenable to GC analysis using liquid injection. Instead, the headspace GC-MS method described herein was developed to quantify the alcohol content of those types of NBA samples. All samples were homogenized and diluted with water prior to analysis. The linear range for the method was 0.05-2% alcohol by volume (ABV) with a coefficient of determination (r^2) > 0.999. Intra- and inter-day repeatability and reproducibility were verified and the use of deuterated ethanol as an internal standard ensured that the method was both robust and relatively insensitive to matrix effects. The new headspace method has been demonstrated to be accurate and precise and can be used for the determination of alcohol content in NBA products.

Introduction

Currently, samples which are unsuited for GC injection are distilled (Figure 1) and then analyzed by densitometry to determine the alcohol content. These samples often contain high levels of sugars, proteins, and/or gums or other gelating agents which, combined with a relatively low ethanol content, render distillation a messy and time-consuming process. Eliminating the need for distillation of NBA samples was a prime motivating factor for development of a static headspace GC-MS (HS-GC-MS) method.

Figure 1. Ice cream samples being distilled.

The static headspace technique is based on the partitioning of analyte between the sample and the headspace vapor in a sealed vial. Under equilibrium conditions, the partition coefficient is constant and proportional to the analyte concentration in the sample. The proportionality is affected by other components in the solution and the vapor, so careful matrix-matching or use of an internal standard is necessary. For example, *tert*-butanol and *n*-propanol have been used as internal standards for the determination of ethanol in blood [1,2] and methanol has been used for analysis of residual alcohol in cooked foods [3]. Deuterated ethanol and *tert*-butanol were tested as internal standards for this method due to the potential for methanol and *n*-propanol to be present in NBA samples. The method was most robust and least susceptible to matrix effects when using deuterated ethanol (*d6*).

Results

Calibration

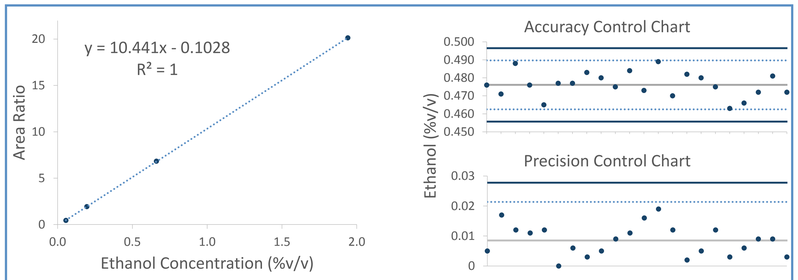

Figure 2. Calibration curve and LCS 1 control charts. Calibration curve is average of 11 replicate measurements, with 95% confidence interval error bars smaller than dot size. For control charts gray lines represent averages, dotted lines represent warning limits, and solid lines represent action limits.

Accuracy

Replicate analysis of a certified reference standard (1.268 %v/v) yielded a recovery of 100.2% with RSD of 5.4%, indicating that the calibration is accurate.

The average spike recovery for 91 NBA samples was 102 ± 10%, indicating that the method is relatively insensitive to matrix effects.

A paired *t*-test comparing distillation to HS-GS-MS for 50 NBA samples showed no difference between the methods at the 95% confidence level. The mean difference and SD was 0.06 ± 0.31 %v/v (median difference -0.03 %v/v).

Precision

Table 1. Repeatability (n=10) and intermediate precision (n=22) results for two laboratory control samples.

		Repeatability		Intermediate Precision	
LCS	Conc (%v/v)	SD (%v/v)	% RSD	SD (%v/v)	% RSD
1	0.476	0.008	1.7	0.007	1.4
2	0.099	0.003	2.6	0.006	6.4

Robustness

Variable	Normal	Low	High
shaker setting [/min]	5 [71]	3 [36]	7 [136]
injector temp (°C)	200	180	220
vial temp (°C)	70	65	75
loop temp (°C)	85	80	90
transfer line temp (°C)	95	90	100
vial equilibration (min)	10	5	15
injection time (min)	0.5	0.3	0.7
vial pressure (psi)	20	16	24

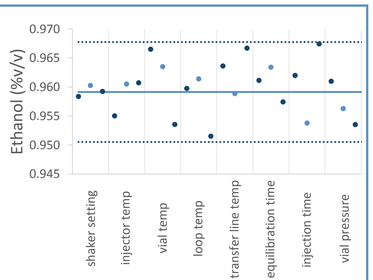

Figure 1. Robustness parameters and measured effects on ethanol determination. Points for each parameter appear in order of low, normal, high and indicated range represents 95% confidence interval for normal points (light blue). The mean and SD (0.959 ± 0.004 %v/v) for all points is identical to those for the normal only points.

Conclusions

The newly developed static headspace GC-MS method for quantitation of ethanol in NBA products has been demonstrated to be accurate, precise, robust, and insensitive to the sample matrix. This method is a suitable replacement for the existing distillation/densitometry method.

Future Work

Reproducibility trials utilizing multiple chemists remain to be performed. There is also interest in expanding the scope of the method to include beverage samples such as kombucha and crème liqueurs. Preliminary results suggest that this method may be a suitable replacement for distillation for those beverages.

Experimental Methods

Standard Preparation

Calibration standards, laboratory control samples (LCS), and spiking solution were prepared by diluting 200-proof ethanol with deionized water. All solutions were analyzed by densitometry (DMA-5000, Anton-Paar) to verify ethanol concentrations as %v/v at 60 °F, taking into account AOAC table 913.02.

Sample Preparation for HS-GC-MS

A 5-50 g portion of solid or highly viscous sample was combined with 10-100 g water, depending on sample size and desired dilution factor, and homogenized using a blender or homogenizer (Fisher Scientific 150). A 1-5 mL portion of liquid sample was combined with 1-20 mL water. One mL aliquots of homogenized samples were weighed into 20-mL headspace vials and 100 µL ISTD (1 ppm ethanol-*d6*) and an additional 100 µL of either water or spike solution was added.

HS-GC-MS

Analyses were carried out using a 7890a GC coupled with an 5975 MSD detector and 7697A headspace sampler (Agilent). See Table 2 for additional method parameters.

Distillation of Samples

A 25-50 g portion of solid or highly viscous sample was combined with 100-200 g water, homogenized (as for samples, above), and 100 mL collected by distillation. A 100 mL portion of liquid sample was combined with 50-70 mL water and 100 mL collected by distillation. Distilled samples were analyzed by densitometry (as for standards, above).

Table 2. HS-GC-MS method parameters.

Column:	J&W DB-624, 30 m × 0.25 mm I.D. × 1.4 µm film thickness
Carrier Gas:	Helium, 1 mL/min constant flow
Temperature:	45 °C for 3 min; 10 °C/min to 60 °C; 25 °C/min to 240 °C for 3 min
Injector / MS Inlet:	200 °C; 200:1 split / 240 °C
Injection:	50 µL loop; 0.5 s
HS Temperature:	Vial 70 °C; Loop 85 °C; Line 95 °C
HS Equilibration:	10 min; shaker setting 5 (71 min^{-1}); vial pressure 20 psi

References

1) C.L. O'Neal, et al., *Forensic Sci. Int.*, 83, **1996**, 31-38.
2) I.A. Wasfi, et al., *J. Chromatogr. B*, 799, **2004**, 331-336.
3) J. Ryapushkina, et al., *Int J. Gastron. Food Sci.*, 5-6, **2016**, 17-26.