Improved Analysis of Flavor Compounds In Scotch Whiskey Using An Aqueous-Stable Polyethylene Glycol Stationary Phase

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Reproducible GC analysis of the congener profile of a distilled spirit is a reliable, objective way of determining quality and mitigating potential adulteration. The high water content of many spirits presents challenges for GC analysis; these challenges however, can be overcome through the use of GC columns and methods that address issues common with aqueous samples.

Introduction

During wine and distilled spirit fermentation, compounds called congeners are formed. These congeners can contribute to a spirit's flavor, but can be harmful if consumed in excess. Some spirits, such as vodka, undergo extra processing steps to eliminate these compounds. Beyond health concerns, an overabundance of a specific congener can signify a problem with production or improper storage conditions. Distilleries also commonly perform congener profile analyses to mitigate adulteration claims and test for authenticity.

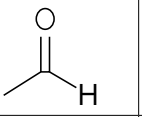
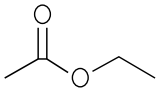
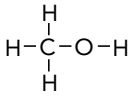
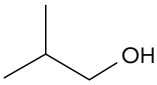
Because the congener profile of a distilled spirit is significant for both quality control and health safety, accurate analysis of these compounds is very important. Testing methods used to analyze these compounds must therefore be both qualitative, quantitative, and reproducible. GC/FID analysis of common congeners (such as those in **Table 1**) is known for its reproducibility and accuracy and is heralded as the industry standard. Polyethylene glycol (PEG) columns have historically provided acceptable selectivity but have been unstable with aqueous samples, resulting in poor reproducibility and decreased lifetime. Traditional analysis is challenging because finished products are composed of 40 and 80 percent water, and congeners are present only in low parts per million (ppm).

Headspace sampling can eliminate some matrix effects and enhance the performance of the more volatile congeners, but will suppress the response of less volatile analytes which may be responsible for unique flavors. Direct injection is therefore still required to verify specific samples. This work explores the separation of distillation congeners on a Zebron™ ZB-WAX*PLUS*™, a water-stable PEG phase.

Materials and Methods

Analyses were performed using an Agilent® 6890 (Agilent Technologies, Palo Alto, CA, USA). Liquid injections used an Agilent liquid autosampler. Headspace samples used an HT-200 Automatic Headspace Sampler (Overbrook Scientific, Boston, MA, USA). All standards are > 95 % purity, and wine and distilled samples were purchased from local grocery stores. Instrument conditions for each method are included with the chromatogram.

Table 1.
Common Distilled Spirit Congeners

			
Acetaldehyde	Ethyl acetate	Methanol	Isobutanol

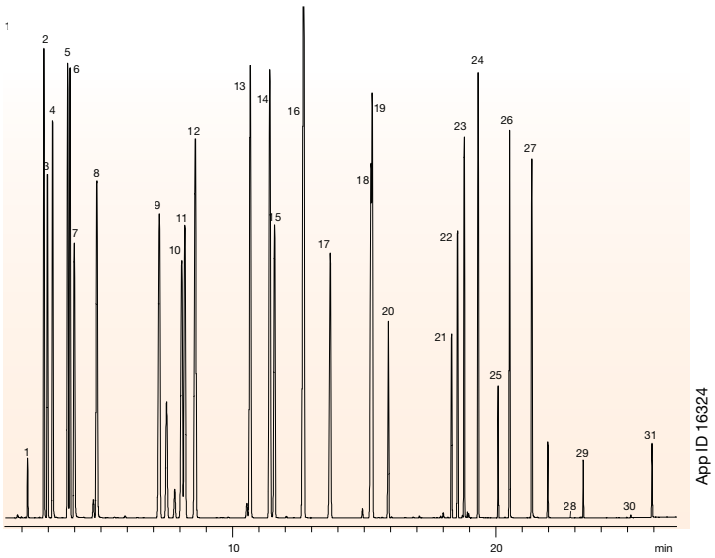
Results and Discussion

Some of the primary congeners are very volatile and may be easily determined using headspace injection. A headspace injection of main congeners and flavor compounds is presented in **Figure 1**. This helps to keep most of the water and contaminants out of the system, which can contribute to decreased chromatographic performance and result in premature column deterioration. The earlier eluting peaks give excellent responses and can easily be quantified. Baseline resolution was achieved for acetaldehyde, ethyl acetate, and methanol (important components in monitoring the distillation process).

In some analyses, it is important to focus on the later eluting compounds because these have a large impact on the complicated flavors of fermented beverages. These congeners form as a result of the conditions of storage and aging and must be monitored to ensure product consistency. These later eluting compounds have lower volatility, and are better analyzed via liquid injections. A liquid injection of the same flavor standard is injected in **Figure 2**. Notice that the later eluting compounds have higher responses given the same concentration. This allows for a more accurate analysis of the flavor compounds which may be unique to a particular brand. For this reason, liquid injections are the preferred method for determining flavors.

On many PEG-based WAX columns, water can affect system performance and reproducibility. Any excess phase that is not crosslinked can bleed in the presence of aqueous compounds like water, creating excess noise and potentially reducing method sensitivity. The ZB-WAX*PLUS* column used employed a deactivation and phase bonding process that resulted in good performance for the water-based sample tested. A schematic of the bonding process is shown in **Figure 3**.

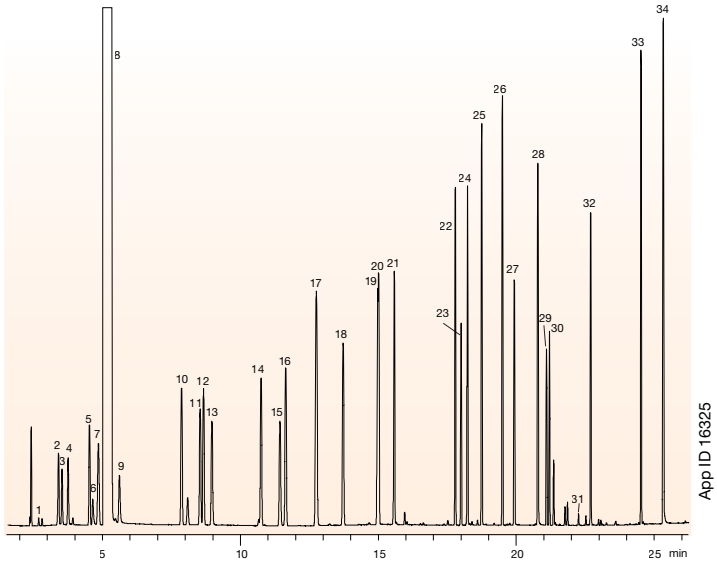
Figure 1.
Distilled Alcohol Standard by Headspace GC/FID



Column: Zebron™ ZB-WAX*PLUS*™
Dimensions: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G013-11
Injection: Split 25:1 @ 210 °C, 200 mL
Carrier Gas: Hydrogen @ 1 mL/min (constant flow)
Oven Program: 35 °C for 7 min to 60 °C @ 5 °C/min for 2 min to 210 °C @ 10 °C/min
Detector: FID @ 230 °C
Note: Static headspace injection (80 °C for 20 min)
Sample:

1. Acetaldehyde
2. Isobutanal
3. Ethyl formate
4. Acrolein
7. Methanol
8. Ethanol
9. Isobutyl acetate
10. 2-Butanol
11. Ethyl butyrate
12. 1-Propanol
13. Isobutanol
14. Allyl alcohol
15. Isoamyl acetate
18. Methyl-2-butanol
19. Methyl-3-butanol
21. Ethyl heptanoate
22. Ethyl lactate
23. Hexanol
24. cis-3-Hexenol
25. Ethyl caprylate
26. Furfural
27. Benzaldehyde
28. Ethyl caprate
29. Diethyl succinate
30. Ethyl laurate
31. Phenyl-2-ethanol

Figure 2.
Distilled Alcohol Standard by Liquid Injection



Column: Zebron ZB-WAX*PLUS*
Dimensions: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G013-11
Injection: Split 25:1 @ 210 °C, 1 µL
Carrier Gas: Hydrogen @ 1 mL/min (constant flow)
Oven Program: 35 °C for 6 min to 60 °C @ 5 °C/min for 2 min to 210 °C @ 10 °C/min
Detector: FID @ 230 °C
Note: 200 ppm standard in methylene chloride
Sample:

1. Acetaldehyde
2. Isobutanal
3. Ethyl formate
4. Acrolein
5. Ethyl acetate
6. Acetal
7. Methanol
8. Methylene chloride
9. Ethanol
10. Isobutyl acetate
11. 2-Butanol
12. Ethyl butyrate
13. 1-Propanol
14. Isobutanol
15. Allyl alcohol
16. Isoamyl acetate
17. 1-Butanol
18. 4-Methyl-2-pentanol
19. Methyl-2-butanol
20. Methyl-3-butanol
21. Ethyl caproate
22. Ethyl heptanoate
23. Ethyl lactate
24. Hexanol
25. cis-3-Hexenol
26. Ethyl caprylate
27. Furfural
28. Benzaldehyde
29. Linalool
30. Linalyl acetate
31. Ethyl caprate
32. Diethyl succinate
33. Ethyl laurate
34. 2-Phenyl ethanol

In **Figure 4**, multiple injections of neat Scotch whiskey were made. Though the whisky sample consisted of ~60 % water, no changes in peak shape or retention times were observed (replicate injections were less than 5 % RSD).

In addition to providing aqueous stability, ZB-WAX*PLUS* also provides very low activity for acidic compounds. This allowed for the fatty acids (eluting past 12 min) to be analyzed within the same run. The lack of acetic acid in the sample suggests that the product was well stored prior to opening and that the cork seal from the bottle was not compromised.

Additional beverages that have not been distilled can also be analyzed using the ZB-WAX*PLUS*. A chromatogram for an Italian wine is shown in **Figure 5**. In this instance, sample preparation consisted of only filtering before injecting. This chromatogram shows baseline separation of early eluting congeners, which can be used to monitor the fermentation process.

Figure 3.
Bonding and deactivation of 100 % aqueous stable column.

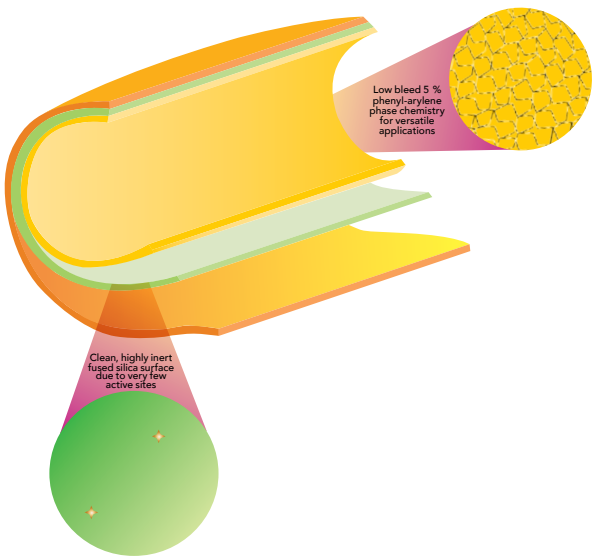


Figure 4.
Replicate Liquid Injections of Undiluted Scotch Whiskey

Column: Zebron™ ZB-WAX*PLUS*™
Dimensions: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G013-11
Injection: Split 30:1 @ 140 °C, 0.2 µL
Carrier Gas: Helium @ 1.4 mL/min (constant flow)
Oven Program: 35 °C for 5 min to 85 °C @ 10 °C/min to 200 °C @ 25 °C/min for 1 min
Detector: FID @ 200 °C
Sample:
1. Acetaldehyde
2. Ethyl acetate
3. Methanol
4. Ethanol
5. Propanol
6. Isobutanol
7. 2-Methylbutanol
8. 3-Methylbutanol

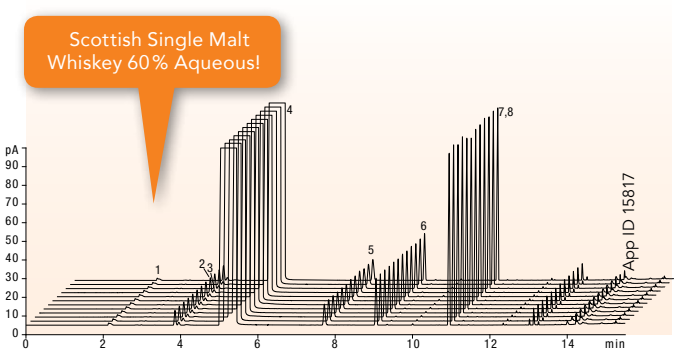
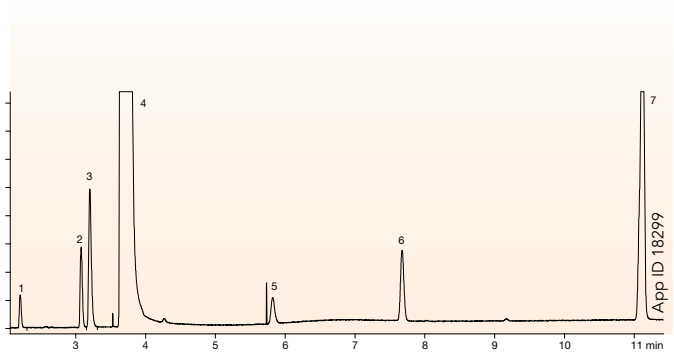


Figure 5.
Filtered Liquid Injection of Italian Wine

Column: Zebron ZB-WAX*PLUS*
Dimensions: 30 meter x 0.32 mm x 0.25 µm
Part No.: 7HM-G013-11
Injection: Split 10:1 @ 150 °C, 0.2 µL
Carrier Gas: Helium @ 2.3 mL/min (constant flow)
Oven Program: 40 °C for 5 min to 150 °C @ 5 °C/min for 5 min to 220 °C @ 20 °C/min for 2 min
Detector: FID @ 280 °C
Accessories: Phenex™-RC Syringe Filter (AF0-2203-52)
Note: Wine has been filtered through 0.2 µm regenerated cellulose filter and directly injected.
Sample:
1. Acetaldehyde
2. Ethyl acetate
3. Methanol
4. Ethanol
5. Propanol
6. Isobutanol
7. 3-Methyl-1-butanol



Conclusion

Method reproducibility and accuracy for distilled spirit analysis is very important for both quality control and health safety. Therefore, using an aqueous stable GC column is the best approach for congener analysis as it allows direct injection. Fermented beverages including distilled spirit congeners have historically been difficult to analyze by direct injection, but can be analyzed successfully using the Zebron ZB-WAX*PLUS* GC column. By using a Zebron ZB-WAX*PLUS* GC column for distilled spirit analysis, accuracy and reproducibility can be achieved without sacrificing resolution.

APPLICATIONS

Ordering Information

Zebron™ ZB-WAX*PLUS*™ GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
10-Meter			
0.10	0.10	20 to 250/260	7CB-G013-02
15-Meter			
0.25	0.25	20 to 250/260	7EG-G013-11
0.53	1.00	20 to 230/240	7EK-G013-22
20-Meter			
0.18	0.18	20 to 250/260	7FD-G013-08
30-Meter			
0.25	0.25	20 to 250/260	7HG-G013-11
0.25	0.50	20 to 250/260	7HG-G013-17
0.32	0.25	20 to 250/260	7HM-G013-11
0.32	0.50	20 to 250/260	7HM-G013-17
0.32	1.00	20 to 230/240	7HM-G013-22
0.53	1.00	20 to 230/240	7HK-G013-22
60-Meter			
0.25	0.15	20 to 250/260	7KG-G013-05
0.25	0.25	20 to 250/260	7KG-G013-11
0.25	0.50	20 to 250/260	7KG-G013-17
0.32	0.25	20 to 250/260	7KM-G013-11
0.32	0.50	20 to 250/260	7KM-G013-17
0.53	1.00	20 to 230/240	7KK-G013-22

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G013-11-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

guarantee

If Zebron GC columns do not provide you with equivalent separations as compared to any other GC column of the same phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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