Distilled Spirit Analysis Using the 100 % Aqueous Stable Zebron™ ZB-WAXPLUS™ GC Column

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During the fermentation process in the production of wines and distilled spirits, compounds called congeners are formed. Some common congeners are listed in Table 1. The congeners add flavor, but can be harmful if consumed in excess. Some spirits, such as vodka, undergo extra processing steps to eliminate these compounds. Aside from health concerns, an overabundance of a specific congener can signify a problem with production or improper storage conditions.

Because the congener profile of a distilled spirit is significant for both quality control and health safety reasons, the ability to analyze these compounds accurately is very important. Therefore, the testing method used to analyze these compounds must be both qualitative, quantitative, and reproducible. Gas Chromatography analysis using Flame Ionization Detection (GC-FID) is known for its reproducibility and accuracy and therefore is the industry standard.

WAX columns provide optimal selectivity for many aqueous soluble compounds, such as the congeners found in alcoholic beverages. However, traditional polyethylene glycol (PEG) phases are unstable with aqueous samples, resulting in poor reproducibility and premature column deterioration. The analysis of distilled spirits is challenging because the finished product is 40-80 % water and congener levels are in the low parts per million (ppm).

Headspace sampling can eliminate some of the matrix effects. This technique can enhance the performance of the more volatile congeners, but will suppress the response of less volatile analytes which may be responsible for unique flavors. Therefore, a direct injection is still required to verify specific samples.

This work explores the separation of distillation congeners on a Zebron ZB-WAXPLUS, a water-stable PEG-based phase.
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**Table 1. Common Distilled Spirit Congeners**
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 chromatogram are included with the chromatogram.
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 chromatographic 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 congeners 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 for each brand. For this reason, liquid injections are the preferred method for determining flavors.

On other PEG-based WAX columns, water can affect system performance and reproducibility. The Zebron ZB-WAXPLUS has been designed to be stable with aqueous samples. This can be seen in Figure 3, where repeated injections were made of a Scotch whiskey consisting of ~60 % water. There is no change in peak shape or retention times over time, and repeated injections have <5 % RSD.

In addition to providing aqueous stability, the Zebron ZB-WAXPLUS 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-WAXPLUS. A chromatogram for an Italian wine is shown in Figure 4. 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.

Results and Discussion
Figure 1. Distilled Alcohol Standard by Headspace GC

Column: Zebron ZB-WAX
Dimensions: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G013-11
Injection: Split 25:1 @ 210 °C, 200 µL
Carrier Gas: Hydrogen @ 1 mL/min (constant flow)
Oven Program: 30 °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
5. Ethyl acetate
6. Acetal
7. Methanol
8. Ethanol
9. Isobutyl acetate
10. 2-Butanol
11. Ethyl butyrate
12. 1-Propanol
13. Isobutanol
14. Allyl alcohol
15. Isoamyl acetate
16. Butanol
17. 4-Methyl-2-pentanol
18. Methyl-2-butanol
19. Methyl-3-butanol
20. Ethyl caproate
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-WAXPLUS  
**Dimensions:** 30 meter x 0.25 mm x 0.25 µm  
**Part No.:** 7HG-G013-11  
**Injection:** Split 25:1 @ 210 °C, 200 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
**Figure 3. Replicate Liquid Injections of Undiluted Scotch Whiskey**

- **Column:** Zebron ZB-WAXPLUS
- **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 200 °C @ 30 °C/min for 1 min
- **Detector:** FID @ 280 °C
- **Note:** Undiluted Scotch whiskey (injected multiple times for reproducibility)

**Sample:**

1. Acetaldehyde
2. Ethyl acetate
3. Methanol
4. Ethanol
5. Propanol
6. Isobutanol
7. 2-Methylbutanol
8. 3-Methylbutanol
Figure 4. 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

**Note:** Wine sample filtered through 0.2 µm RC filter and directly injected

**Sample:**
1. Acetaldehyde
2. Ethyl acetate
3. Methanol
4. Ethanol
5. Propanol
6. Isobutanol
7. 3-Methyl-1-butanol
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-WAXPLUS GC column.

By using a Zebron ZB-WAXPLUS GC column for distilled spirit analysis, accuracy and reproducibility can be achieved without sacrificing resolution.